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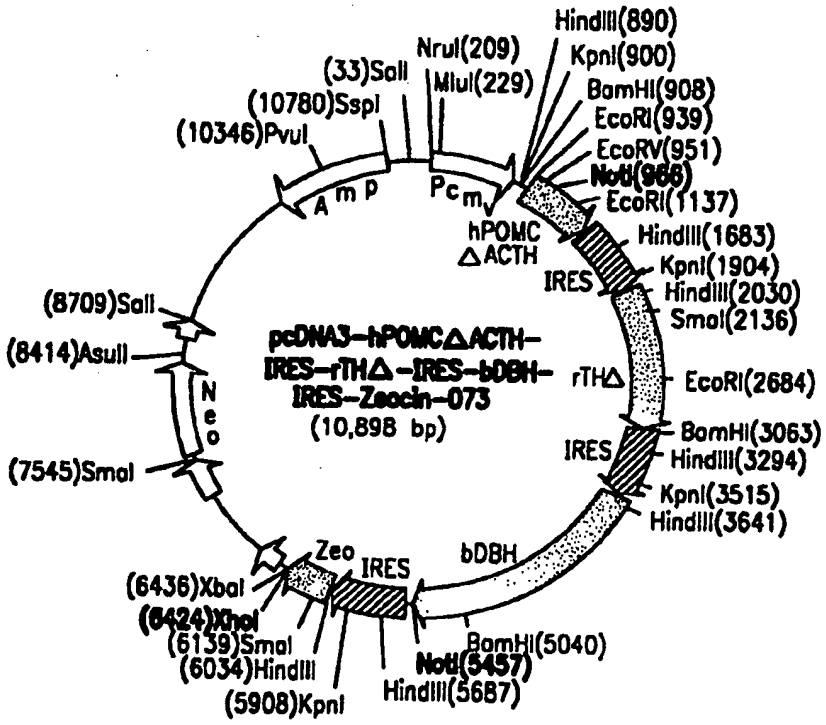
(54) Title: CELL LINE PRODUCING ANALGESIC COMPOUNDS FOR TREATING PAIN

(57) Abstract

A genetically engineered cell line that produces at least one catecholamine, at least one endorphin, and at least one enkephalin, for the treatment of pain. The cells may be provided directly to a patient in need thereof, or encapsulated to form a bioartificial organ.



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# Cell line producing analgesic compounds for treating pain

## Field of the Invention

The present invention relates to a cell line useful for the treatment of pain. More particularly, the cell line of this invention has been genetically engineered to produce at least one analgesic compound from each of the groups consisting of endorphins, enkephalins, and catecholamines.

## Background of the Invention

Pain is a common symptom of disease. The superficial dorsal horn of the spinal cord, where primary afferent fibers carrying nociceptive information terminate, contains enkephalinergic interneurons and high densities of opiate receptors. In addition, there is a dense concentration of noradrenergic fibers in the superficial laminae of the spinal cord.

Acute pain arises in response to acute noxious stimuli. Chronic pain is predominantly due to neuropathies of central or peripheral origin. This

neuropathic pain is the result of aberrant somatosensory processing that can result in increased sensitivity to a painful stimulus (hyperalgesia) and pain associated with a stimulus that does not usually provoke pain (allodynia).

Intrathecal injection of morphine into the spinal subarachnoid space produces potent analgesia. Similarly, intrathecal administration of norepinephrine or noradrenergic agonists also produces analgesia. See, e.g., Sagen et al., Proc. Natl. Acad. Sci. USA, 83, pp. 7522-26 (1986).

Co-administration of subeffective doses of opiates, such as enkephalins, and catecholamines, such as norepinephrine, may synergize to produce analgesia. Ibid. Chromaffin cells in the adrenal medulla produce and release several neuroactive substances including norepinephrine, epinephrine, met-enkephalin, leu-enkephalin, neuropeptide Y, vasoactive intestinal polypeptide, somatostatin, neurotensin, cholecystokinin and calcitonin gene-related peptide. See, e.g., Sagen et al., Proc. Natl. Acad. Sci. USA, 83, pp. 7522-26 (1986); Sagen et al., Jour. Neurochem., 56, pp. 623-27 (1991).

Because chromaffin cells produce both opioid peptides and catecholamines, one approach to reduction of nociceptive response or pain sensitivity has investigated transplanting adrenal medullary tissue, as well as isolated adrenal chromaffin cells, directly into CNS pain modulatory regions, in attempts to provide analgesia. See, e.g., Sagen et al., Brain Research, 384, pp. 189-94 (1986); Vaguero et al., Neuroreport, 2, pp. 149-51 (1991); Ginzberg and

Seltzer, Brain Research, 523, pp. 147-50 (1990); Sagen et al., Pain, 42. pp. 69-79 (1990).

Attempts to produce analgesic have been made using both allogeneic and xenogeneic chromaffin tissue or cells transplants. Allograft tissue is in limited supply, and is not readily available, particularly for in human pain treatment programs. In addition, allogeneic human tissue carries the risk of pathogenic contamination. See e.g., Hama and Sagen, Brain Research, 651, pp. 183-93 (1994).

Xenogeneic donors may provide large quantities of material that can be readily obtained. For this reason, bovine adrenal tissue has been used. See, e.g., Hama and Sagen, Brain Research, 651, pp. 183-93 (1994).

However, potentially serious host consequences, as well as ultimate graft rejection, are inherent problems in transplantation between disparate species. Complete graft rejection of whole or dissociated tissue may occur even in the CNS, normally thought to be immunologically privileged, due to presence of highly antigenic cells in the xenografts, particularly endothelial cells. In addition, the donor tissue must be carefully screened to avoid introduction of viral contaminants, or other pathogens, to the host. To overcome graft rejection, immunosuppression is required typically using cyclosporine A.

Some reduction in pain sensitivity has been reported resulting from these transplants, particularly for the reduction of low intensity chronic pain. In most reports, significant differences between control and transplanted animals were noted only after nicotine

administration to stimulate opioid peptide production. However, there have been some reports that analgesia has been observed in a rat chronic pain model from basal level activity of chromaffin tissue allografts. See, e.g., Vaquero et al., NeuroReport, 2, pp. 149-51 (1991) and Hama and Sagen, Brain Research, 651, pp. 183-93 (1994).

Bovine adrenal chromaffin cells have been encapsulated to form a bioartificial organ ("BAO") for implantation into rats for the treatment of acute and chronic pain. See, e.g., Sagen et al., J. Neurosci., 13, pp. 2415-23 (1993) and Hama et al., 7th World Congress Pain, Abstract 982, Paris France (1993). Initial trials in human subject have been conducted using encapsulated bovine chromaffin cells. See, Aebischer et al., Transplantation, 58, pp. 1275-77 (1994).

There have also been attempts to induce antinociception using other cells, e.g., AtT-20 cells. AtT-20 cells were originally derived from a mouse anterior pituitary tumor. These cells synthesize and secrete ß-endorphin. See, e.g., Wu et al., J. Neural Transpl. & Plasticity, 5, pp. 15-26 (1993). AtT-20/hENK cells are AtT-20 cells that have been genetically engineered to carry the entire human pro-enkephalin A gene (i.e. containing 6 met-enkephalin sequences and one leu-enkephalin sequence) with 200 bases of 5'-flanking sequence and 2.66 kilobases of 3'-flanking sequence. See Wu et al., supra, Comb et al., EMBO J., 4, pp. 3115-22 (1985).

Wu et al., J. Neural Transpl. & Plasticity, 5, pp. 15-26 (1993) refers to rat hosts transplanted

with AtT-20 or AtT-20/hENK cells. Unstimulated AtT-20/hENK cells produced more antinociception (tail flick test) than produced by AtT-20 implants. In contrast, isoproterenol stimulation produced more antinociception with AtT-20 cells than with AtT-20/hENK cells. Ibid.

In mice hosts, AtT-20 or AtT-20/hENK implants did not affect basal response to thermal nociceptive stimuli. Mice receiving AtT-20 implants developed tolerance to ß-endorphin and a μ-opioid agonist (DAMGO). Mice receiving AtT-20/hENK implants developed tolerance to an δ-opioid agonist (DPDPE). In response to repeated doses of an μ opiate agonist, mice receiving AtT-20/hENK implants developed less tolerance compared to mice receiving AtT-20 cells or controls.

The antinociceptive effect of isoproterenol treatment appeared equal in mice receiving AtT-20 or AtT-20/hENK cell implants. See, Wu et al., J. Neuroscience, 14, pp. 4806-14 (1994). Wu et al. speculated that one reason for the absence of additional antinociception in mice implanted with enkephalin producing AtT-20/hENK cells may be due to lack of sensitivity of the behavioral assays. Another possible reason was that met-enkephalin's known antagonist effect on morphine induced antinociception offset the potentiating effect of the single leu-enkephalin, particularly since there are 6 met-enkephalin sequences for each leu-enkephalin sequence in pro-enkephalin A.

Summary of the Invention

The present invention provides a cell line that has been genetically engineered to produce at least one analgesic compound from each of the groups consisting of endorphins, enkephalins, and catecholamines. The cell line may be used in the treatment of pain.

There are advantages to using a cell line over the use of primary cells. Expensive and time consuming testing to ensure safety and performance criteria for cells must be performed for individual isolations of primary cells. Less testing is required of a cell bank. There is no need to isolate primary cells. Output of the desired analgesics may be more stable since the performance of primary cells may be dependent on the age, sex, health or hormonal status of the donor animal. It is also possible to achieve higher output of the desired products, as well as to engineer specifically modified peptides into the cell line. This permits delivery of multiple analgesics simultaneously. Expression of one or more of the analgesics can be regulated (by using a regulatable promoter to drive expression). In addition, for safety, a "suicide" gene can be incorporated into the cell line. Further, for encapsulation purposes proliferating cells have the advantage that they divide to replace dying or dead cells.

Brief Description of the Drawing

Figure 1 is a plasmid map of vector pBS-hPOMC-027, pBS-IgSP-hPOMC-028 and pBS-IgSP-hPOMC-ΔACTH-029.

Figure 2 is a plasmid map of vectors pCEP4-hPOMC-030, pCEP4-hPOMC-031, pcDNA3-hPOMC-034 and pcDNA3-hPOMC-035.

Figure 3 is a plasmid map of vectors pCEP4-hPOMC-ΔACTH-032, pCEP4-hPOMC-ΔACTH-033, pcDNA3-hPOMC-ΔACTH-36 and pcDNA3-hPOMC-ΔACTH-037.

Figure 4 is a plasmid map of vectors pcDNA3-rTH-044, pcDNA3-rTHΔ-045, and pcDNA3-rTHDKS-075 (also represented as pcDNA3-rTHΔKS-075).

Figure 5 is a plasmid map of vectors pcDNA3-rTHΔ-IRES-bDBH-088 and pcDNA3-rTHΔKS-IRES-bDBH-076.

Figure 6 is a plasmid map of vector pZeo-Pcmv-rTHΔKS-IRES-bDBH-088.

Figure 7 is a plasmid map of vector pBS-Pcmv-rTHΔIRES-bDBH-067.

Figure 8 is a plasmid map of vector pBS-hPOMC-ΔACTH-IRES-rTHΔIRES-bDBH-068.

Figure 9 is a plasmid map of vector pcDNA3-hPOMC-ΔACTH-IRES-rTHΔ-IRES-bDBH-069.

Figure 10 is a plasmid map of vector pcDNA3-IRES-Zeocin-072.

Figure 11 is a plasmid map of vector pcDNA3-hPOMC-ΔACTH-IRES-rTHΔ-IRES-bDBH-IRES-Zeocin-073.

Figure 12 is a plasmid map of vector pcDNA3-hPROA+KS-091.

Detailed Description of the Invention

In order that this invention may be more fully understood, the following detailed description is set forth.

Any suitable cell may be transformed with the recombinant DNA molecules of this invention. Among the contemplated cells are chromaffin cells, including conditionally immortalized chromaffin cells such as those described in WO 96/02646, Neuro-2A, PC12, PC12a, SK-N-MC, AtT-20, and RIN cells including RINa and RINb. Preferably the cell has endogenous prohormone convertases and/or dopa decarboxylases.

SK-N-MC cells, a neuroepithelioma cell line, co-expresses several neuropeptides, including enkephalin, cholecystokinin and gastrin-releasing peptide. See, e.g., Verbeeck et al., J. Biol. Chem., 265, pp. 18087-090 (1990). The pro-enkephalin A gene has been expressed in SK-N-MC cells. See, e.g., Folkesson et al., Mol. Brain Res., 3, pp. 147-54 (1988). We prefer AtT-20 and RIN cells, most preferably RIN cells.

RIN cells are a pancreatic endocrine cell line derived from rat. See, e.g., Horellou et al., J. Physiol., 85, pp. 158-70 (1991). RIN cells are known to endogenously produce GABA and ß-endorphin.

Some of the characteristics of various contemplated cells are shown in Table 1.

Table 1

| Cells | Analgesic Substances | Other Components |
|---|---|---|
| Chromaffin | NE, met-enkephalin | TH, DDC, DβH, PC |
| PC12, PC12a | low NE & met-enkephalin | DDC, DβH, PC |
| AtT-20 | β-endorphin | DDC, PC |
| RINa | β-endorphin, GABA | DDC, PC |
| RINb | β-endorphin | DDC, PC |
| Neuro 2A | | DDC, DβH, PC |

TH = Tyrosine hydroxylase converts tyrosine - l-dopa
DDC = Dopamine decarboxylase converts l-dopa - dopamine (DA)
DβH = Dopamine β-Hydroxylase converts DA - norepinephrine (NE)
PC = Prohormone Convertases process POMC to β-endorphin and Pro-enkephalin A (ProA) to met-enkephalin.
AtT20 = Mouse pituitary corticotroph cell line that endogenously secretes β-endorphin via expression of Pro-opiomelanocortin (POMC).
RIN = Rat insulinoma
Neuro 2A = Mouse neuroblastoma

The primary delivery products include at least one each of an endorphin, an enkephalin and a catecholamine.

Enkephalins and endorphins are endogenous opioid peptides in humans. These opioid peptides comprise approximately 15 compounds ranging from 5 to 31 amino acids. These compounds bind to and act at least in part via the same μ opioid receptor as morphine, but are chemically unrelated to morphine. In addition, these compounds stimulate other opiate receptors. Yaksh and Malmberg, Textbook of Pain, 3rd Ed. (Eds. P. Wall and R. Melzack), "Central Pharmacology of Nociceptive Transmission," pp. 165-200, 1994 (New York).

The opioid peptides have common chemical properties, but are synthesized in different pathways.

ß-endorphin, the most abundant endorphin, is synthesized as part of a larger precursor molecule, pro-opiomelanocortin ("POMC"). The POMC molecule contains the full sequence of adrenocorticotrophic hormone ("ACTH"), α-melanocyte-stimulating hormone ("α-MSH"), ß-MSH, and ß-lipotropin. The POMC precursor molecule also has the potential to generate other endorphins, including α-endorphin and gamma-endorphin. Processing of the POMC precursor occurs differently within various tissues according to the localization of cleavage enzymes, such as prohormone convertases, within those tissues.

In the pituitary, POMC is cleaved to produce ACTH and ß-endorphin, and the ACTH is not further processed. In contrast, in the hypothalamus, ACTH is converted to ß-MSH. While different cell types may synthesize the same primary gene product, the final profile of hormone secretion may differ widely.

This invention contemplates use of a DNA sequence encoding any suitable endorphin that has analgesic activity. In addition, analogs or fragments of these endorphins that have analgesic activity are also contemplated. Thus the endorphin to be produced by the cells of this invention may be characterized by amino acid insertions, deletions, substitutions and modifications at one or more sites in the naturally occurring amino acid sequence of the desired endorphin. We prefer conservative modifications and substitutions (i.e., those having a minimal effect on the secondary or tertiary structure of the endorphin and on the analgesic properties of the endorphin). Such conservative substitutions include those described by

Dayhoff in Atlas of Protein Sequence and Structure, 5, (1978) and by Argos, Embo J., 3, pp. 779-85 (1989).

Techniques for generating such variants of naturally occurring endorphins are well known. For example, codons in the DNA sequence encoding the wild type endorphin may be altered by site specific mutagenesis.

This invention contemplates using a DNA sequence encoding the entire POMC precursor molecule. This embodiment takes advantage of the host cell's cleavage enzymes (i.e., Prohormone convertase 2) to generate a suite of endorphins, some or all of which may have analgesic properties.

This invention also contemplates use of DNA fragments of the POMC gene that encode a particular desired endorphin.

The DNA and amino acid sequence of POMC are well known. Cochet et al., Nature, 297, pp. 335-9 (1982); Takahashi et al., Nucl. Acids Res., 11, pp. 6847-58 (1983).

We prefer a DNA sequence encoding POMC in which the ACTH coding region has been deleted. The preferred endorphin encoded by this construct is ß-endorphin.

Some enkephalins are synthesized in the adrenal glands as part of a large protein, pro-enkephalin A, that contains six repeats of the Met-enkephalin sequence and one Leu-enkephalin structure. Met-enkephalin, as well as Met-enkephalin-Arg-Phe and Met-enkephalin-Arg-Gly-Leu have significant antinociceptive activity. See, e.g., Sagen et al., Brain Res., 502, pp. 1-10 (1989).

Other enkephalins, i.e., dynorphins and neo-endorphins are derived from a distinct molecule, pro-enkephalin B. Additional "cryptic" peptides are also encoded within the structure of these precursor proteins, and may be released by "pro-hormone-type" cleavage. See, e.g., Harrison's "Principles Of Internal Medicine", 12th Edition, pp. 1168-69 (1991).

This invention contemplates use of a DNA sequence encoding any suitable enkephalin that has analgesic activity. Analogs and active fragments that have analgesic properties are also contemplated. Such analogs or fragments may thus have amino acid insertions, deletions, substitutions at one or more sites in the naturally occurring amino acid sequence. Such variants may be generated as described above.

This invention contemplates use of a DNA sequence encoding a desired enkephalin in its "mature" form. In addition, this invention contemplates using a DNA sequence encoding the entire pro-enkephalin A precursor, or the entire pro-enkephalin B precursor. Further, we also contemplate using DNA encoding a fusion, or fragment of these sequences, that upon expression yields one or more enkephalin-like molecules that have analgesic properties.

We prefer use of a DNA sequence encoding the entire pro-enkephalin A precursor molecule. The DNA and amino acid sequence of pro-enkephalin A are well known. Folkesson, supra. This embodiment takes advantage of the host cell's cleavage enzymes, such as prohormone convertase, to generate a suite of enkephalins, some or all of which may have analgesic

properties. The preferred enkephalin encoded by this construct is Met-enkephalin.

There are three naturally occurring catecholamines which function as neurotransmitters in the central nervous system; norepinephrine ("NE"), epinephrine ("E"), and dopamine. NE is associated with postganglionic sympathetic nerve endings. NE exerts its effects locally in the immediate vicinity of its release.

Catecholamines are synthesized from the amino acid tyrosine, which is sequentially hydroxylated to form dihydroxyphenylalanine (dopa), decarboxylated to form dopamine, and then hydroxylated on the beta position of the side chain by dopamine beta hydroxylase to form NE. Harrison's, supra, pp. 380. NE is N-methylated to E by phenylethanolamine-N methyltransferase ("PNMT").

Hydroxylation of tyrosine by tyrosine hydroxylase ("TH") is the rate limiting step in NE synthesis. Regulation of dopa and NE synthesis in the adrenal medulla may be accomplished by changes in the amount and the activity of TH.

In addition, regulation of synthesis of E from NE may occur by changes in the amount and the activity of phenylethanolamine-N-methyltransferase ("PNMT"). PNMT is inducible by glucocorticoids from the adrenal cortex. Ibid.

Catecholamines are maintained in high concentration in adrenal medullary chromaffin tissue, mostly as E. Opioid peptides are also stored in the adrenal gland.

NE and E have similar affinities at $\alpha_2$ receptors and therefore both potentially contribute to analgesia. Bylund, FASEB J., 6, PP. 832-39 (1992). The enkephalin peptides that predominantly include met-enkephalin selectively activate delta (δ) opioid receptors. Reisine and Bell, Trends Neurosci., 16, pp. 506-10 (1993). Activation of $\alpha_2$ adrenergic and δ opioid receptors in the spinal cord each result in antinociception and are potentially synergistic. Yaksh and Malmberg, Progress in Pain Research and Management, Vol. 1, Ed. Fields and Lisbeskind, IASP Press, Seattle, pp. 141-71 (1994). Activation of δ versus (μ) opioid receptors in experimental animals results in fewer adverse side effects including constipation and addiction liability (Lee et al., J. Pharmacol. Exp. Ther., 267, pp. 883-87 (1993). The combined delivery of different opioidergic and adrenergic agents may decrease the magnitude of tolerance that develops to a single agent and lead to sustained pain relief. Yaksh and Reddy, Anesthesiol., 54, pp. 451-67 (1981).

This invention contemplates use of a DNA sequence encoding catecholamine biosynthetic enzymes or analogs or fragments thereof to obtain catecholamines that have analgesic properties. The preferred catecholamines in this invention are NE and E.

In one embodiment, the host cell is transformed with the genes necessary to accomplish production of NE or E, as desired. The selection of heterologous gene sequences required depends upon the complement of catecholamine synthesizing enzymes normally occurring in the host cell. For example, RIN cells, and AtT-20 cells lack tyrosine hydroxylase

("TH") and dopamine beta hydroxylase ("DBH"). However, RIN and AtT-20 cells contain endogenous dopa decarboxylase ("DDC"). If the desired catecholamine is E, then the gene encoding PNMT is also required. The gene encoding PNMT is known. Baetge et al., Proc. Nat'l Acad. Sci., 83, pp. 5455-58 (1986).

The gene encoding TH is known. See, e.g., United States patent 5,300,436, incorporated herein by reference. Modified TH variants are also known. United States patent 5,300,436. In addition, truncated versions of TH that contain the necessary C-terminal catalytic domains are also known. See, e.g., Daubner et al., Protein Science, 2, pp. 1452-60 (1993).

AtT-20 cells have been transformed with wild type TH, as well as various TH muteins. See, e.g., Wu et al., J. Biol. Chem., 267, pp. 25754-758 (1992).

The sequence of the DBH gene is also well known. See, e.g., Lamoroux et al., EMBO J., 6, pp. 3931-37 (1987).

It will be appreciated that in addition to the preferred DNA sequences described herein, there will be many degenerate DNA sequences that code for the desired analgesics.

Secondary compounds with potential analgesic action may also be produced by the cells of this invention. Such compounds include galanin and somatostatin. In addition, neuropeptide Y, neurotensin and cholecystokinin may be produced by the transformed cells of this invention. The cells of this invention may normally produce some or all of these compounds, or may be genetically engineered to do so using standard techniques.

Standard methods may be used to obtain or synthesize the genes encoding the analgesic compounds to be produced by the cells of this invention.

For example, the complete amino acid sequence of the desired compound may be used to construct a back-translated gene. A DNA oligomer containing a nucleotide sequence coding for the desired analgesic compound may be synthesized. For example, several small oligonucleotides coding for portions of each desired polypeptide may be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for assembly.

The DNA sequence encoding each desired analgesic compound, may or may not also include DNA sequences that encode a signal sequence. Such signal sequence, if present, should be one recognized by the cell chosen for expression of the analgesic compound. It may be prokaryotic, eukaryotic or a combination of the two. It may also be the signal sequence of the native compound. It generally is preferred that a signal sequence be encoded and most preferably that the native signal sequence be used.

Once assembled, the DNA sequences encoding the desired compounds will be inserted into one or more expression vectors and operatively linked to expression control sequences appropriate for expression in the desired transformed cell.

Proper assembly may be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in the transformed cell. As is well known in the art, in order to obtain high expression levels of a transfected

gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression cell.

The choice of expression control sequence and expression vector will depend upon the choice of cell. A wide variety of expression host/vector combinations may be employed. Useful expression vectors for eukaryotic hosts, include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus.

We prefer pcDNA3, pCEP4, pZeoSV (InVitrogen, San Diego) and pNUT.

Any of a wide variety of expression control sequences may be used in these vectors. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Examples of useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating system and other sequences known to control the expression of genes of eukaryotic cells or their viruses, and various combinations thereof.

It should of course be understood that not all vectors and expression control sequences will function equally well to express the DNA sequences described herein. Neither will all cells function equally well with the same expression system. However, one of skill in the art may make a selection among

these vectors, expression control sequences and cells without undue experimentation. For example, in selecting a vector, the host cell must be considered because the vector must replicate in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, should also be considered.

In selecting an expression control sequence, a variety of factors should also be considered. These include, for example, the relative strength of the sequence, its controllability, and its compatibility with the actual DNA sequence encoding the desired analgesic compounds, particularly as regards potential secondary structures. Host cells should be selected by consideration of their compatibility with the chosen vector, the toxicity of the product coded for by the DNA sequences, their secretion characteristics, their ability to fold the polypeptides correctly, and their culture requirements. If the host cell is to be encapsulated, cell viability when encapsulated and implanted in a recipient should also be considered.

Within these parameters, one of skill in the art may select various vector/expression control sequence/host combinations that will express the desired DNA sequences in culture.

In one embodiment, cells (e.g., RIN cells) are sequentially transformed with 4 separate expression vectors containing the POMC gene, the pro-enkephalin A gene, the TH gene and the DBH gene. In such a transformed host cell, amplification of copy number of the heterologous genes is more difficult to achieve.

Thus use of fewer expression vectors is preferred. Most preferably, a single expression vector, containing all 4 heterologous genes, is used.

In a particular embodiment RIN cells are sequentially transformed with 3 expression vectors. The first vector contains the POMC gene operably linked to the CMV promoter. Preferably a truncated version of the POMC gene is used, having the ACTH coding region deleted. The second vector contains the pro-enkephalin A gene operably linked to the CMV promoter. Preferably the proA construct contains the Kozak sequence immediately upstream of the start codon. The third vector contains both the TH gene (preferably truncated and having the Kozak consensus sequence immediately upstream of the start codon) and the DBH gene. In this embodiment, the TH gene is operably linked to the CMV promoter. The DBH gene is operably linked to an internal ribosome entry site promoter sequence. RIN cells are then transformed sequentially with each expression vector according to known protocols.

In another embodiment, a single expression vector containing the pro-enkephalin A gene, the POMC gene, the TH gene, and the DBH gene is constructed. Preferably, the ACTH region of the POMC gene is deleted. Preferably the TH gene is truncated.

Multiple gene expression from a single transcript is preferred over expression from multiple transcription units. One approach for achieving expression of multiple genes from a single eukaryotic transcript takes advantage of sequences in picorna viral mRNAs known as internal ribosome entry sites ("IRES"). These sites function to facilitate protein

translation from sequences located downstream from the first AUG of the mRNA.

Macejak and Sarnow reported that the 5' untranslated sequence of the immunoglobulin heavy chain binding protein (BiP, also known as CRP 78, the glucose-regulated protein of molecular weight 78,000) mRNA can directly confer internal ribosome binding to an mRNA in mammalian cells, in a 5'-cap independent manner, indicating that translation initiation by an internal ribosome binding mechanism is used by this cellular mRNA. Nature 353, pp. 90-94 (1991).

WO 94/24870 refers to use of more than two IRES for translation initiation from a single transcript, as well as to use of multiple copies of the same IRES in a single construct.

This invention also contemplates use of a "suicide" gene in the transformed cells. Most preferably, the cell carries the TK (thymidine kinase) gene as a safety measure, permitting the host cell to be killed in vivo by treatment with gancyclovir.

Use of a "suicide" gene is known in the art. See, e.g., Anderson, published PCT application WO 93/10218; Hamre, published PCT application WO 93/02556. The recipient's own immune system provides a first level of protection from adverse reactions to the implanted cells. If encapsulated, the polymer capsule itself may be immuno-isolatory. The presence of the TK gene (or other suicide gene) in the expression construct adds an additional level of safety to the recipient of the implanted cells.

Preferred vectors for use in this invention include those that allow the DNA encoding the analgesic

compounds to be amplified in copy number. Such amplifiable vectors are well known in the art. They include, for example, vectors able to be amplified by DHFR amplification (see, e.g., Kaufman, United States Patent 4,470,461, Kaufman and Sharp, "Construction Of A Modular Dihydrafolate Reductase cDNA Gene: Analysis Of Signals Utilized For Efficient Expression", Mol. Cell. Biol., 2, pp. 1304-19 (1982)) or glutamine synthetase ("GS") amplification (see, e.g., United States patent 5,122,464 and European published application 338,841). Such amplification can be used to increase output of the desired analgesic compounds.

Other techniques for increasing the output of the desired analgesic compounds are contemplated. For example, subcloning existing polyclonal cell lines is contemplated. Cells are cloned by limiting dilution to a single cell in each well. Cell clones are cultures, and the clones are tested to select the clone with the highest output of analgesic substances.

Another technique for increasing the output of the desired analgesic compounds involves cloning altered forms of biosynthetic enzymes with higher activity than the wild type form (i.e., the truncated TH 1-155). Some truncated forms of TH have 4-6 times increased activity over the wild type form of TH. See, e.g., Daubner et al., "Expression and characterization of catalytic and regulatory domains of rat tyrosine hydroxylase" Protein Science, 2, pp. 1452-60 (1993).

In addition, use of tyrosine-free media to select to increase tetrahydrobiopterin cofactor levels may potentially increase tyrosine hydroxylase activity. See, e.g., Horellou et al., "Retroviral transfer of a

human tyrosine hydroxylase cDNA in various cell lines; regulated release of dopamine in mouse anterior pituitary AtT-20 cells", Proc. Natl. Acad. Sci. USA, 86, pp. 7233-37 (1989).

Preferably, the output of ß-endorphin ranges between 1 and 10,000 pg/$10^6$ cells/hr. Preferably, the output of met-enkephalin ranges between 1 and 10,000 pg/$10^6$ cells/hr. Preferably, the output of catecholamines ranges between 1 and 1,000 pmoles/$10^6$ cells/hr.

The cells of this invention may be implanted into a mammal, including a human, for the treatment of pain. If implanted unencapsulated, any suitable implantation protocol may be used, including those outlined by Sagen et al., United States patent 4,753,635, incorporated herein by reference.

It may be desirable to encapsulate the genetically modified cells of this invention before implantation. Such encapsulated cells form a bioartificial organ ("BAO"). BAOs may be designed for implantation in a recipient or can be made to function extra-corporeally. The BAOs useful in this invention typically have at least one semipermeable outer surface membrane or jacket surrounding a cell-containing core. The jacket permits the diffusion of nutrients, biologically active molecules and other selected products through the BAO. The BAO is biocompatible.

In some cases, the membrane may serve to also immunoisolate the cells by blocking the cellular and molecular effectors of immunological rejection. The use of immunoisolatory membranes allows for the implantation of allo and xenogeneic cells into an

individual without the use of immunosuppression. If biologically active molecules are released from the isolated cells, they pass through the surrounding semipermeable membrane into the recipient's body. If metabolic functions are provided by the isolated cells, the substances to be metabolized enter the BAO from the recipient's body through the membrane to be acted on by the cells.

A variety of types of membranes have been used in the construction of BAOs. Generally, the membranes used in BAOs are either microporous or ultrafiltration grade membranes. A variety of membrane materials have been suggested for use in BAOs, including PAN/PVC, polyurethanes, polysulfones, polyvinylidienes, and polystyrenes. Typical membrane geometries include flat sheets, which may be fabricated into "sandwich" type constructions, having a layer of living cells positioned between two essentially planar membranes with seals formed around the perimeter of the device. Alternatively, hollow fiber devices may be used, where the living cells are located in the interior of a tubular membrane. Hollow fiber BAOs may be formed step-wise by loading living cells in the lumen of the hollow fiber and providing seals on the ends of the fiber. Hollow fiber BAOs may also be formed by a coextrusion process, where living cells are coextruded with a polymeric solution which forms a membrane around the cells.

BAOs have been described, for example, in United States patent Nos. 4,892,538, 5,106,627, 5,156,844, 5,158,881, and 5,182,111, and PCT Application Nos. PCT/US/94/07015, WO 92/19195, WO

93/03901, and WO 91/00119, all of which are incorporated herein by reference.

BAOs may contain other components that promote long term survival of the encapsulated cells. For example, WO 92/19195 refers to implantable immunoisolatory biocompatible vehicles having a hydrogel matrix for enhancing cell viability.

The encapsulating membrane of the BAO may be made of a material which is the same as that of the core, or it may be made of a different material. In either case, a surrounding or peripheral membrane region of the BAO which is permselective and biocompatible will be formed. The membrane may also be constructed to be immunoisolatory, if desired. The core contains isolated cells, either suspended in a liquid medium or immobilized within a hydrogel matrix.

The choice of materials used to construct the BAO is determined by a number of factors and is described in detail in Dionne WO 92/19195. Briefly, various polymers and polymer blends can be used to manufacture the capsule jacket. Polymeric membranes forming the BAO and the growth surfaces therein may include polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones, polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

BAOs may be formed by any suitable method known in the art. One such method involves coextrusion of a polymeric casting solution and a coagulant which

can include biological tissue fragments, organelles, or suspensions of cells and/or other therapeutic agents, as described in Dionne, WO 92/19195 and United States Patents 5,158,881, 5,283,187 and 5,284,761, incorporated herein by reference.

The jacket may have a single skin or a double skin. A single-skinned hollow fiber may be produced by quenching only one of the surfaces of the polymer solution as it is co-extruded. A double-skinned hollow fiber may be produced by quenching both surfaces of the polymer solution as it is co-extruded.

Numerous capsule configurations, such as cylindrical, disk-shaped or spherical are possible.

The jacket of the BAO will have a pore size that determines the nominal molecular weight cut off (nMWCO) of the permselective membrane. Molecules larger than the nMWCO are physically impeded from traversing the membrane. Nominal molecular weight cut off is defined as 90% rejection under convective conditions. In situations where it is desirable that the BAO is immunoisolatory, the membrane pore size is chosen to permit the particular factors being produced by the cells to diffuse out of the vehicle, but to exclude the entry of host immune response factors into the BAO. Typically the nMWCO ranges between 50 and 200 kD, preferably between 90 and 150 kD. The most suitable membrane composition will also minimize reactivity between host immune effector molecules known to be present at the selected implantation site, and the BAO's outer membrane components.

The core of the BAO is constructed to provide a suitable local environment for the particular cells

isolated therein. The core can comprise a liquid medium sufficient to maintain cell growth. Liquid cores are particularly suitable for maintaining transformed cell lines like PC12 cells. Alternatively, the core can comprise a gel matrix. The gel matrix may be composed of hydrogel (alginate, "Vitrogen™", etc.) or extracellular matrix components. See, e.g., Dionne WO 92/19195.

Compositions that form hydrogels fall into three general classes. The first class carries a net negative charge (e.g., alginate). The second class carries a net positive charge (e.g., collagen and laminin). Examples of commercially available extracellular matrix components include Matrigel™ and Vitrogen™. The third class is net neutral in charge (e.g., highly crosslinked polyethylene oxide, or polyvinylalcohol).

Any suitable method of sealing the BAO may be used, including the employment of polymer adhesives and/or crimping, knotting and heat sealing. These sealing techniques are known in the art. In addition, any suitable "dry" sealing method can also be used. In such methods, a substantially non-porous fitting is provided through which the cell-containing solution is introduced. Subsequent to filling, the BAO is sealed. Such a method is described in copending United States application Serial No. 08/082,407, herein incorporated by reference.

One or more in vitro assays are preferably used to establish functionality of the BAO prior to implantation in vivo. Assays or diagnostic tests well known in the art can be used for these purposes. See,

e.g., Methods In Enzymology, Abelson [Ed], Academic Press, 1993. For example, an ELISA (enzyme-linked immunosorbent assay), chromatographic or enzymatic assay, or bioassay specific for the secreted product can be used. If desired, secretory function of an implant can be monitored over time by collecting appropriate samples (e.g., serum) from the recipient and assaying them. If the recipient is a primate, microdialysis may be used.

The number of BAOs and BAO size should be sufficient to produce a therapeutic effect upon implantation is determined by the amount of biological activity required for the particular application. In the case of secretory cells releasing therapeutic substances, standard dosage considerations and criteria known to the art are used to determine the amount of secretory substance required. Factors to be considered are discussed in Dionne, WO 92/19195.

Implantation of the BAO is performed under sterile conditions. Generally, the BAO is implanted at a site in the host which will allow appropriate delivery of the secreted product or function to the host and of nutrients to the encapsulated cells or tissue, and will also allow access to the BAO for retrieval and/or replacement. The preferred host is a primate, most preferably a human.

A number of different implantation sites are contemplated. These implantation sites include the central nervous system, including the brain, spinal cord, and aqueous and vitreous humors of the eye. Preferred sites in the brain include the striatum, the cerebral cortex, subthalamic nuclei and nucleus Basalis

of Meynert. Other preferred sites are the cerebrospinal fluid, most preferably the subarachnoid space and the lateral ventricles. This invention also contemplates implantation into the kidney subcapsular site, and intraperitoneal and subcutaneous sites, or any other therapeutically beneficial site.

In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only, and are not to be construed as limiting the scope of this invention in any manner.

Examples

Construction of Polycistronic Expression Vectors

**Construction of IgSP-POMC Fusion**

The SmaI-SalI fragment containing the human POMC exon 3 was subcloned into pBS cloning vector (Stratagene). See Takahashi, supra; Cochet, supra. The resulting plasmid was named as pBS-hPOMC-027. See Fig. 1.

A PCR fragment was generated using two oligonucleotide primers, termed oCNTF-003 (SEQ ID NO: 1) and oIgSP-018, (SEQ ID NO: 2) and the pNUT plasmid containing the human CNTF gene. See Baetge et al., Proc. Natl. Acad. Sci. USA, 83, pp. 5454-58 (1986). Both primers oCNTF-003 and oIgSP-018, contain synthetic BamHI and SmaI restriction sites, respectively, at the 5' ends.

The 196 base pair (bp) PCR fragment was digested with restriction endonucleases BamHI and the SmaI-isoschizomer XmaI, and electrophoresed through an

1% SeaPlaque agarose. The 193 bp HindIII/XmaI DNA fragment was excised and purified using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME).

pBS-hPOMc-027 was also digested with BamHI and XmaI and purified from 1% SeaPlaque agarose using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME). The ligation mixture was transformed into E. coli DH5α (Gibco BRL, Gaithersburg, MD).

Positive sub-clones were initially identified by the cracking gel procedure (Promega Protocols and Applications Guide, 1991). Minilysate DNA was then prepared using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME) and subject to BamHI and SmaI restriction digestions. The positive sub-clone was named as pBS-IgSP-hPOMC-028. See Fig. 1. The nucleotide sequence of the fusion junction in pBS-IgSP-hPOMC-028 was determined by the dideoxynucleotide sequence determination using the Sequenase kit (USBC, Cleveland). The sequence of the IgSP-hPOMC fusion is shown in SEQ ID NO: 3.

**Construction of IgSP-POMC Expression Vectors**

The IgSP-hPOMC DNA fragment in pBS-IgSP-hPOMC-028 was subcloned into pcDNA3 (Invitrogen Corp., San Diego, CA) and pCEP4 (Invitrogen Corp., San Diego, CA) in sense and anti-sense orientations.

The NotI-SalI IgSP-hPOMC fragment from pBS-IgSP-hPOMC-028 was ligated with the NotI-XhoI digested pCEP4 resulting in the sense orientation clone named as pCEP4-hPOMC-030. Fig. 2. The BamHI-SalI IgSP-hPOMC fragment from pBS-IgSP-hPOMC-028 was ligated with the

BamHI-XhoI digested pCEP4 resulting in the anti-sense orientation clone named as pCEP4-hPOMC-031. Fig. 2. The insert orientation in pCEP4-hPOMC-030 and -031 was confirmed by BamHI, NotI, SalI and NotI/SalI restriction digestions as well as by dideoxynucleotide sequence determination using the Sequenase kit (USBC, Cleveland).

The BamHI-SalI IgSP-hPOMC fragment from pBS-IgSP-hPOMC-028 was ligated with the BamHI-XhoI digested pcDNA3 resulting in the sense orientation clone named as pcDNA3-hPOMC-034. Fig. 2. The NotI-HindIII IgSP-hPOMC fragment from pBS-IgSP-hPOMC-028 was ligated with the NotI-HindIII digested pcDNA3 resulting in the anti-sense orientation clone named as pcDNA3-hPOMC-035. Fig. 2. Restriction digestion using SmaI, BamHI, EcoRI, and BamHI/EcoRI was used to confirm the insert orientation in pcDNA3-hPOMC-034, whereas HindIII, NotI and SalI were used for pcDNA3-hPOMC-035.

**Construction of ACTH Deleted IgSP-POMC**

The ACTH coding region in the POMC gene in pBS-IgSP-hPOMC-028 was deleted. pBS-IgSP-hPOMC-028 was first digested with XmaI restriction enzyme and treated with pfu DNA polymerase (Promega, Madison, WI). The XmaI-pfu DNA polymerase treated pBS-IgSP-hPOMC-028 was then digested with StuI restriction enzyme and purified from 1% SeaPlaque agarose using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME). The self-ligation mixture was transformed into E. coli DH5α (Gibco BRL, Gaithersburg, MD). Positive sub-clones were identified by BamHI/HindIII restriction digestion and named as pBS-IgSP-hPOMCΔACTH-029. See Fig. 1. The

nucleotide sequence of the ACTH deletion region in pBS-IgSP-hPOMC-ΔACTH-029 was confirmed by the dideoxynucleotide sequence determination. The sequence of the IgSP-hPOMC-ΔACTH fusion is shown in SEQ ID NO: 4.

**Construction of ACTH Deleted IgSP-POMC Expression Vectors**

The IgSP-hPOMC-ΔACTH DNA fragment in pBS-IgSP-hPOMC-ΔACTH-029 was subcloned into pcDNA3 (Invitrogen Corp., San Diego, CA) and pCEP4 (Invitrogen Corp., San Diego, CA) in sense and anti-sense orientations. The NotI-SalI IgSP-hPOMC-ΔACTH fragment from pBS-IgSP-hPOMC-ΔACTH-029 was ligated with the NotI-XhoI digested pCEP4 resulting in the sense orientation clone named as pCEP4-hPOMC-ΔACTH-032 (Fig. 3). The BamHI-SalI IgSP-hPOMC-ΔACTH fragment from pBS-IgSP-hPOMC-ΔACTH-029 was ligated with the BamHI-XhoI digested pCEP4 resulting in the anti-sense orientation clone named as pCEP4-hPOMC-ΔACTH-033 (Fig. 3). The insert orientation in pCEP4-hPOMC-ΔACTH-032 and -033 was confirmed by BamHI and EcoRI restriction digestions as well as by dideoxynucleotide sequence determination using the Sequenase kit (USBC, Cleveland).

The BamHI-SalI IgSP-hPOMC-ΔACTH fragment from pBS-IgSP-hPOMC-ΔACTH-029 was ligated with the BamHI-XhoI digested pcDNA3 resulting in the sense orientation clone named as pcDNA3-hPOMΔACTH-036 (Fig. 3). The NotI-HindIII IgSP-hPOMC-ΔACTH fragment from pBS-IgSP-hPOMC-ΔACTH-029 was ligated with the NotI-HindIII

digested pcDNA3 resulting in the anti-sense orientation clone named as pcDNA3-hPOMC-ΔACTH-037 (Fig. 3).

Restriction digestion using PvuII and EcoRI was used to confirm the insert orientation in pcDNA3-hPOMC-ΔACTH-036, whereas SalI and EcoRI were used for pcDNA3-hPOMC-ΔACTH-037.

**Cloning of Full Length and Truncated TH cDNA**

Total RNA from PC12 cells was prepared using the guanidinium thiocyanate-based TRI reagent (Molecular Research Center, Inc., Cincinnati, OH). Five hundred ng of PC12 total RNA was reverse transcribed at 42°C for 30 minutes in a 20µl reaction volume containing 10 mM Tris.HCl (pH 8.3), 50 mM KCl, 4 mM of each dNTP, 5 mM $MgCl_2$, 1.25 µM oligo (dT) 15-mer, 1.25 µM random hexamers, 31 units of RNase Guard RNase Inhibitor (Pharmacia, Sweden) and 200 units of SuperScript II reverse transcriptase (Gibco BRL, Gaithersburg, MD). Two micro-liters of the above reverse transcribed cDNA was added to a 25 µl PCR reaction mixture containing 10 mM Tris.HCl (pH 8.3), 50 mM KCl, 800 of each nM dNTP, 2 mM MgC12, 400 nM of primers #1 and #2, and 2.5 units of Thermus aquaticus (Taq) DNA polymerase (Boehringer Mannheim, Germany).

To generate the full length TH cDNA, oligonucleotide primers orTH-052 (SEQ ID NO: 5) and orTH-053 (SEQ ID NO: 6) were used. For the truncated TH, primers orTH-054 (SEQ ID NO: 7) and orTH-053 (SEQ ID NO: 6) were used instead. These oligonucleotides were constructed based on published TH sequence information in Grima et al., Nature, 326, pp. 707-11 (1987); US patent 5,300,436, and Daubner, supra.

Primers orTH-052 (SEQ ID NO: 5) and orTH-054 (SEQ ID NO: 7) have synthetic HindIII restriction site at the 5' end where orTH-053 has BamHI at the 5' end. The PCR reaction mixtures were subject to 30 amplification cycles consisted of: denaturation, 94°C 30 seconds (first cycle 2 minutes); annealing, 50°C 1 minute; and extension, 72°C 3.5 minutes (last cycle 5 minutes). The 1537 bp full length and 1087 bp truncated rat TH PCR fragments were digested with restriction endonucleases BamHI and HindIII and resolved on an 1% SeaPlaque agarose gel. The 1531-bp and 1081-bp HindIII/BamHI DNA fragments were excised and purified using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME).

pcDNA3 expression vector was also digested with BamHI and HindIII and purified from 1% SeaPlaque agarose using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME). The ligation mixture was transformed into E.coli DH5α (Gibco BRL, Gaithersburg, MD).

Cracking gel procedure (Promega Protocols and Applications Guide, 1991) was used to screen out the positive sub-clones. The identity of the correct clones was further verified by BamHI/HindIII double digestion.

The positive sub-clones for the full-length and truncated rat TH in pcDNA3 were named as pcDNA3-rTH-044 (Fig. 4) and pcDNA3-rTHΔ-045 (Fig. 4), respectively. The nucleotide sequence of both full-length and truncated rat TH PCR clones was determined by the dideoxynucleotide sequence determination using

the Sequenase kit (USBC, Cleveland). The sequence of the rTHΔ construct is shown in SEQ ID NO: 16.

To optimize the translation efficiency of the truncated rat TH, oligonucleotide primer orTH-078 (SEQ ID NO: 8) was designed so that the consensus Kozak sequence is immediate up stream to the start codon ATG. pcDNA3-rTHΔ-45 was used as the template in a 50 µl PCR reaction mixture with reagent composition identical to the one described above with the exception that the oligonucleotide primers were replaced with orTH-078 (SEQ ID NO: 8) and orTH-053 (SEQ ID NO: 6). The 1097 bp PCR product was cloned into pcDNA3 in the same manner as described above. The resulting sub-clone was named pcDNA3-rTHΔKS-75 (Fig 4). The sequence of the rTHΔKS construct is shown in SEQ ID NO: 17.

**Construction of rTH-IRES-bDBH Fusion Gene**

Recombinant PCR methodology was used to generate the rTH-IRES-bDBH fusion gene. Oligonucleotides oIRES-057 (SEQ ID NO: 9) and obDBH-065 (SEQ ID NO: 10) are specific for IRES and bDBH gene sequences, respectively, and contain synthetic BamHI and NotI restriction sites at the 5' end, respectively. Oligonucleotides oIRES-bDBH-064 (SEQ ID NO: 11) and oIRES-bDBH-066 (SEQ ID NO: 12) are complementary to each other. Furthermore, oligonucleotide primer oIRES-bDBH-064 (SEQ ID NO: 11) has its 5' 16 nucleotides identical to the IRES sequence and its 3' 18 nucleotides identical to the bDBH sequence; and vice versa for oIRES-bDBH-066 (SEQ ID NO: 12).

Two first PCR reactions were carried out using oligonucleotide pairs oIRES-057/oIRES-bDBH-066

and oIRES-bDBH-064/obDBH-065 on templates pCTI-001 (with an insert containing the IRES sequence shown in SEQ ID NO: 30) and pBS-bDBH-006 (containing the bovine DBH gene cloned from bovine adrenal chromaffin cells, Lamoroux et al., EMBO J., 6, pp. 3931-37 (1987)) plasmids, respectively. One hundred ng of template DNA was added to a 50 µl PCR reaction mixture containing 10 mM Tris.HCl (pH 8.3), 50 mM KCl, 800 of each nM dNTP, 2 mM MgCl2, 400 nM of primers #1 and #2, and 2.5 units of Thermus aquaticus (Taq) DNA polymerase (Boehringer Mannheim, German).

The PCR reaction mixtures were subject to 30 amplification cycles consisted of: denaturation, 94 °C for 30 seconds (first cycle 2 minutes); annealing, 50 °C 1 minute; and extension, 72 °C 30 seconds (last cycle 5 minutes). The PCR products were resolved on 1% TrivieGel 500 (TrivieGen). Two agarose plugs containing each one of the first PCR products were transfer to a tube containing 50 µl of PCR reaction mixtures identical to the one described above with the exception that the oligonucleotides oIRES-057 and obDBH-065 were used.

The second PCR reaction was subject to 30 amplification cycles consisted of: denaturation, 94 °C for 30 seconds (first cycle 2 minutes); annealing, 60 °C 30 seconds (second to fourth cycles 37 °C 2 minutes); and extension, 72 °C 30 seconds (last cycle 2 minutes). The 2407 bp IRES-bDBH fusion PCR product and the cloning vector pcDNA3-rTHΔ-45 were digested with BamHI and NotI restriction enzymes and subsequently purified from 1% SeaPlaque agarose gel using the FMC

SpinBind DNA purification kit (FMC BioProducts, Rockland, ME).

The ligation of IRES-bDBH/BamHI/NotI and pcDNA3-rTHΔ-045/BamHI/NotI would generate a rTHΔ-IRES-bDBH expression vector named as pcDNA3-rTHΔ-IRES-bDBH-066 (Fig. 5) whereas that of IRES-bDBH/BamHI/NotI and pcDNA3-rTHΔKS-075/BamHI/NotI would generate a rTHΔKS-IRES-bDBH expression vector, named as pcDNA3-rTHΔKS-IRES-bDBH-076 (Fig. 5), where the start codon ATG in rTHΔ is preceded with a consensus Kozak sequence. The sequence of the rTHΔ-IRES-bDBH construct is shown in SEQ ID NO: 18. The sequence of the rTHΔKS-IRES-bDBH construct is shown in SEQ ID NO: 19. The ligation mixture was transformed into DH5α (Gibco BRL, Gaithersburg, MD). The positive clones were identified by the cracking gel procedure (Promega, Madison, WI) and restriction digestions using HindIII, BamHI, HindIII/BamHI, SmaI and NotI.

The 4114 bp NruI-XhoI fragment containing the CMV promoter-rTHΔKS-IRES-bDBH was excised out of pcDNA3-rTHΔKS-IRES-bDBH-076 and subcloned into pZeoSV cloning vector (Invitrogen Corp., San Diego, CA) digested with ScaI and XhoI in the multiple cloning site. The resulting expression vector was named as pZeo-Pcmv-rTHΔKS-IRES-bDBH-088 (Fig. 6).

**Construction of IgSP-hPOMC ACTH-rTHD-IRES-bDBH Fusion Gene**

The 4100 bp NruI-NotI fragment containing the CMV promoter, rTHD-IRES-bDBH fusion gene, and BGH polyadenylation sequence was excised out of pcDNA3-

rTHΔ-IRES-bDBH-066 and subcloned into the pBS (Stratagene, La Jolla, CA) cloning vector.

The resulting plasmid pBS-Pcmv-rTHΔ-IRES-bDBH-067 (Fig. 7) was used as the intermediary construct to which the recombinant PCR IgSP-hPOMCDACTH-IRES fragment would be inserted.

Oligonucleotide oIgSP-068 (SEQ ID NO: 13), containing a synthetic EcoRV restriction site, is specific for the IgSP sequence.

Oligonucleotide primer orTHΔ-073 (SEQ ID NO: 14) is specific for the rTHΔ sequence and contains an endogenous SmaI restriction site.

Oligonucleotide primers ohPOMC-IRES-069 (SEQ ID NO: 15) and ohPOMC-IRES-070 (SEQ ID NO: 20) are complementary to each other. Furthermore, oligonucleotide primer ohPOMC-IRES-069 has its 5', 18 nucleotides identical to the hPOMC sequence and its 3' 12 nucleotides identical to the IRES sequence; and vice versa for ohPOMC-IRES-070.

Oligonucleotide primers oIRES-rTHΔ-071 (SEQ ID NO: 21) and oRIRES-rTHΔ-072 (SEQ ID NO: 22) are complementary to each other. In addition, oligonucleotide primer oIRES-rTHΔ-071 has its 5' 15 nucleotides identical to the rTHΔ sequence and its 3' 18 nucleotide identical to the IRES sequence; and vice versa for oRIRES-rTHΔ-072.

Three sets of first PCR reactions were carried out.

PCR reaction A: template pBS-IgSP-hPOMCDACTH-029, oligonucleotides oTgSP-068/ohPOMC-IRES-069;

PCR reaction B: template pCTI-001, oligonucleotides ohPOMC-IRES-070/oIRES-rTHΔ-071; and

PCR reaction C: template pcDNA3-rTHΔ-045, oligonucleotides orIRES-rTHΔ-072/orTHΔ-073.

The three sets of first PCR reactions were carried in 50 µl PCR reaction mixture containing 100 ng of template DNA, 10 mM Tris. HCl (pH 8.3), 50 mM KCl, 800 of each nM dNTP, 2 mM MgCl23, 400nM of primers #1 and #2, and 2.5 units of Thermus aquaticus (Taq) DNA polymerase (Boehringer Mannheim, Germany).

The PCR reaction mixtures were subject to 30 amplification cycles consisted of: denaturation, 94 °C for 30 seconds (first cycle 2 minutes); annealing, 50 °C 1 minute; and extension, 72 °C 30 seconds (last cycle 5 minutes).

The PCR products were resolved on 1% TrivieGel 500 (TrivieGen). Two agarose plugs containing each one of the PCR products from PCR reactions B and C were transferred to a tube containing 50 µl of PCR reaction mixtures identical to the one described above with the exception that the oligonucleotides ohPOMC-IRES-070 and orTHΔ-073 were used.

The second PCR reaction was subject to 30 amplification cycles consisted of: denaturation, 94 °C for 30 seconds (first cycle 2 minutes); annealing, 60 °C 30 seconds (second to fourth cycles 37 °C 2 minutes); and extension, 72 °C 30 seconds (last cycle 2 minutes).

The PCR products were treated as described above. Agarose plugs containing the PCR products from the second PCR reaction and the PCR reaction A were combined and subjected to a third PCR amplification using oIgSP-068/rTHΔ-073. The 1203 bp IgSP-hPOMC-IRES-

rTHΔ fusion PCR product and the cloning vector pBS-Pcmv-rTHΔ-IRES-bDBH-067 were digested with EcoRV and XmaI restriction enzymes and subsequently purified from 1% SeaPlaque agarose gel using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME). The ligation mixture was transformed into DH5α (Gibco BRL, Gaithersburg, MD).

The positive clones were identified by the cracking gel procedure (Promega, Madison, WI) and restriction digestions using EcoRI, KpnI and NotI. The resulting clone was named as pBS-IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH-068. Fig. 8. The sequence of this construct is shown in SEQ ID NO: 23.

**Construction of IgSP-hPOMCACTH-IRES-rTHΔ-IRES-bDBH Expression Vectors**

The 4491 bp NotI fragment containing the IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH gene was excised out of the pBS-IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH-068 and subcloned into the pcDNA3 (Invitrogen Corp., San Diego, CA) at the NotI site in the multiple cloning site. Restriction digestion using NotI and SmaI confirmed that the IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH gene was inserted in the sense orientation resulting in pcDNA3-IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH-069. See Fig. 9.

**Construction of IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH-IRES-Zeocine Expression Vector**

Recombinant PCR methodology was used to generate the IRES-Zeocine fusion gene. Oligonucleotides oIRES-074 (SEQ ID NO: 24) and oZeocin-

077 (SEQ ID NO: 25) are specific for IRES and Zeocin gene sequences, respectively, and contain synthetic NotI and XhoI restriction sites at the 5' end, respectively. Oligonucleotides oIRES-Zeocin-075 (SEQ ID NO: 26) and oIRES-Zeocin-076 (SEQ ID NO: 27) are complementary to each other. Furthermore, oligonucleotide oIRES-Zeocin-075 has its 5'15 nucleotides identical to the Zeocin sequence and its 3' 18 nucleotides identical to the IRES sequence; and vice versa for oIRES-Zeocin-076.

Two first PCR reactions were carried out using oligonucleotide pairs oIRES-074/oIRES-Zeocin-075 and oIRES-Zeocin-076/oZeocin-075 on templates pCTI-001 and pZeoSV (Invitrogen Corp., San Diego, CA) plasmids, respectively.

One hundred ng of template DNA was added to a 50 µl PCR reaction mixture containing 10mM Tris.HCl (pH 8.3), 50 mM KCl, 800 of each nM dNTP, 2 mM MgCl2, 400 nM of primers #1 and #2, and 2.5 units of Thermus aquaticus (Taq) DNA polymerase (Boehringer Mannheim, Germany).

The PCR reaction mixtures were subject to 30 amplification cycles consisted of: denaturation, 94 °C for 30 seconds (first cycle 2 minutes); annealing, 50 °C 1 minute; and extension, 72 °C 30 seconds (last cycle 5 minutes).

The PCR products were resolved on 1% TrivieGel 500 (TrivieGen). Two agarose plugs containing each one of the first PCR products were transfer to a tube containing 50 µl of PCR reaction mixtures identical to the one described above with the

exception that the oligonucleotides oIRES-074 and oZeocin-077 were used.

The second PCR reaction was subject to 30 amplification cycles consisted of: denaturation, 94 °C for 30 seconds (first cycle 2 minutes); annealing, 50 °C 30 seconds (second to fourth cycles 37 °C 2 minutes); and extension, 72 °C 30 seconds (last cycle 2 minutes).

The 974 bp IRES-Zeocin fusion PCR product and the cloning vector pcDNA3 were digested with NotI and XhoI restriction enzymes and subsequently purified from 1% SeaPlaque agarose gel using the FMC SpinBind DNA purification kit (FMC BioProducts, Rockland, ME).

The ligation of IRES-Zeocin/NotI/XhoI and pcDNA3/NotI/XhoI would generate an intermediate cloning vector named as pcDNA3-IRES-Zeocin-072. Fig. 10.

The positive clones were identified by the cracking gel procedure (Promega, Madison, WI) and restriction digestions using HindIII, SmaI, XhoI, NotI and NotI/XhoI.

To generate the final IgSP-hPOMCDACTH-IRES-rTHD-IRES-bDBH-IRES-Zeocine Expression Vector, a 4491 bp NotI fragment containing the IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH gene was excised out of the pBS-IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH-068 (Fig. 8; SEQ ID NO: 23) and subcloned in to the pcDNA3-IRES-Zeocin-072 (Fig. 10) at the NotI site in the multiple cloning site.

Restriction digestion using NotI and SmaI confirmed that the IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH gene was inserted in the sense orientation resulting in pcDNA3-IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH-IRES-Zeocin-

073. The sequence of this construct is shown in SEQ ID NO: 28. Fig. 11.

**Construction of ProA+KS Fusion**

A construct containing the coding region of the human pro-enkephalin A gene with the consensus Kozak sequence immediately upstream to the start codon ATG. The sequence of this construct is shown in SEQ ID NO: 29.

**Construction of hProA+KS Expression Vector**

The HindIII/BamHI fragment containing the hProA+KS fusion was ligated into BamHI and Hind III digested pcDNA3 expression vector substantially as described above. After screening as described above, a positive sub-clone was named pcDNA3-hProA+KS-091. Fig. 12. Construction of the pBS-CMV Pro A vector is detailed in Mothis, J. and Lindberg, I., Endocrinology, 131, pp. 2287-96 (1992).

**Transformation of Cells**

RIN and AtT-20 cells were transformed as follows.

The RINa and AtT-20 based cell lines were grown in DMEM (Gibco) with 10% fetal bovine serum and pen-strep-fungizone (Gibco) base media. The cells were plated out in P100 petri dishes (750,000 cells/dish) in 10 ml of base media. 18-24 hours later, the cells were transfected using calcium phosphate method with a kit made by Stratagene (San Diego, CA). A 10 µg amount of the plasmid vector DNA was diluted in 450 µl of deionized sterile water. Then, 50 µl of a 10x buffer

(solution #1) was added to the plasmid DNA. A 500 µl amount of solution #2 was immediately added to the DNA containing solution and mixed gently. This was incubated at room temperature for 20 minutes and then the 1.0 ml solution was added to the cells in the petri dish. The cells were incubated overnight and 18-24 hours later the cells were washed 2x with Hanks balanced salt solution without calcium and magnesium. Then, the cells were cultured in base media + selection drugs. The cells were selected in either 600 µg/ml geneticin (Gibco) or 400 µg/ml hygromycin (Boehringer Mannheim) or 500 µg/ml Zeocin (In Vitrogen, San Diego, CA). Cells were sequentially transfected and selected to obtain the final cell line.

The RINa cells were transfected with plasmid pCEP4-hPOMC-030 containing the POMC gene. This is a hygromycin resistant vector. The cells were also transformed with plasmid pcDNA3-hProA+KS-091. This is a geneticin resistant vector. Finally, the cells were transfected with plasmid pZeo-PCMV-rTHΔKS-IRES-bDBH-088 which conferred Zeocin resistance.

The AtT-20 cells were transfected with plasmid pBS-CMV-ProA and pCEP4-POMC-ΔACTH-32 which conferred geneticin and hygromycin resistance, respectively. Finally, the cells were transfected with plasmid pZeo-Pcmv-rTHΔKS-IRES-bDBH-088.

We have tested a number of media for cell growth. Surprisingly we have found that in certain serum-free medias, the above cell lines have enhanced neurotransmitter output, compared to serum-containing media. We prefer CHO-Ultra (Biowhitaker) for the

growth of AtT-20 cells, and Ultra-Culture (Biowhitaker) for the growth of RINa cells.

Output of various analgesics from one transformed RINa cell line (RINa/ProA/P030/P088) is shown in Table 2. All values represent unstimulated cells. Output of ß-endorphin and met-enkephalin is in pg/$10^6$ cells/hr. ß-endorphin and met-enkephalin were measured by radioimmunoassay using Incstar kits (Stillwater, Minnesota). Catecholamine output is in pmoles/$10^6$ cells/hr. The numbers in parentheses represent values from cells that were preincubated 18 hours with 100 µM tetrahydrobiopterin. Catecholamines were measured by high performance liquid chromatography as described in Lavoie et al., "Two PC12 pheochromocytoma lines sealed in hollow fiber-based capsules tonically release l-dopa in vitro", Cell transplantation, 2, pp. 163-73 (1993). GABA output from these RINa cells was 28 ng/$10^6$ cells/hrs.

Table 2

| Cell Line | Endogenous Analgesic Substances | ß-endorphin | Met-enk | DA | E |
|---|---|---|---|---|---|
| RIN a/ ProA/ POMC/ TH-IRES-DßH | ß-endorphin GABA | 22 | 17 | 3 (6) | 0 (2) |

There are encrypted enkephalin fragments which are not fully processed from the pro-enkephalin precursor molecule. These encrypted enkephalins have opioid receptor binding activity. We digested these encrypted enkephalins to measure opioid activity. The trypsin digest protocol is as follows. A 2 µg/ml trypsin (Worthington #34E470) solution is added to media

samples on ice. Samples are vortexed, then incubated for 20 minutes in a 37°C waterbath. After the 20 minute digest, samples are returned to ice and 100 ng/ml carboxypeptidase B (Sigma #C-7011) is added. Samples are mixed by vortexing, and returned to the 37°C waterbath for 15 minutes. Samples are placed on ice once more and 10 ug/ml trypsin inhibitor is added. At this stage, samples are either extracted for met-enkephalin or immediately frozen for future extraction. This results in the full enzymatic cleavage to free all met-enkaphalin from the longer encrypted fragments. A met-enkaphalin radioimmunoassay of the digested sample gives total met-enkaphalin from the supermatant. The transformed RINa cells appear to have greater than 5 fold more encrypted enkaphalins compared to fully processed met-enkaphalin.

Fiber capsule formation and characteristics

Hollow fibers are spun from a 12.5-13.5% poly(acrylonitrile vinylchloride) solution by a wet spinning technique. Cabasso, Hollow Fiber Membranes, vol. 12, Kirk-Othmer Encyclopedia of Chemical Technology, Wiley, New York, 3rd Ed. pp. 492-517 (1980), Unites States patent 5,158,881, incorporated herein by reference.

The resulting membrane fibers may either be double skinned or single skinned PAN/PVC fibers. In order to make implantable capsules, lengths of fiber are first cut into 5 cm long segments and the distal extremity of each segment sealed with an acrylic glue. Encapsulation hub assemblies are prepared by providing lengths of the membrane described above, sealing one

end of the fiber with a single drop of LCM 24 (Light curable acrylate glue, available from ICI), curing the glue with blue light, and repeating the step with a second drop. The opposite end is previously attached to a frangible necked hub assembly, having a silicone septum through which the cell solution may be introduced. The fiber is glued to the hub assembly by applying LCM 22 to the outer diameter of the hub assembly, pulling the fiber up over it, and curing with blue light. The hub/fiber assemblies are placed in sterilization bags and are ETO sterilized.

Following sterilization with ethylene oxide and outgassing, the fibers are deglycerinated by ultrafiltering first 70% EtOH, and then HEPES buffered saline solution through the walls of the fiber under vacuum.

Preparation and Encapsulation of Transformed Cells

The transformed cells are prepared and encapsulated as follows:

A matrix solution is prepared using a commercially available alginate, collagen or other suitable matrix material. The cell solution was diluted in the ratio of two parts matrix solution to one part cell solution containing the transformed cells described above. We prefer Vitrogen (Celtix, Santa Clara) as a matrix for AtT-20 cells.

We prefer Organogen (Organogenesis, Canton, MA) as a matrix for RINa cells. The RINa based cells are prepared for encapsulation by the following method. The cells are grown in base media of DMEM + 10% fetal bovine serum during the proliferation phase. These

cells can be removed from the tissue culture flasks by two washes in Hanks balanced salt solution without calcium and magnesium. Then the cells are incubated in 0.25% trypsin + EDTA for 1 minute. This is removed and the cells are rinsed free of the flask using Hanks balanced salt solution without calcium and magnesium solution. The cells are placed in 10 mls of base media and centrifuged at 100 x g for 2 minutes. The cells are resuspended in 10 mls of the preferred serum free media (Ultra culture, Biowhitaker, Walkersville, MD). Surprisingly, the RINa cells secrete more analgesic substances when cultured in this serum free media relative to serum continuing base media.

The cells are centrifuged at 100 g twice in the preferred serum free media before the cells are concentrated 1:1 with the preferred Organogen matrix. Organogen is a 1% bovine tendon collagen obtained as a sterile solution. 8 parts of this solution are mixed with 1 part 10X DPBS. 0.5 N sodium hydroxide is added until physiological pH is attained (approximately 250 µls).

The final concentration of the cell + matrix solution used for encapsulation can range from 20,000 - 50,000 cells/µl. The cells are counted in a standard manner on a hemocytometer.

The cell/matrix suspension is placed in a 1 ml syringe. A Hamilton 1800 Series 50 microliter syringe is set for a 15 microliter air bubble, is inserted into a 1 ml syringe containing the cell solution and 30 microliters are drawn up. The cell solution is injected through the silicone seal of the hub/fiber assembly into the lumen of a modacrylic

hollow fiber membrane with a molecular weight cutoff of approximately 50,000-100,000 daltons. Ultrafiltration should be observed along the entire length of the fiber. After one minute, the hub is snapped off the sub-hub, exposing a fresh surface, unwet by cell solution. A single drop of LCM 24 is applied and the adhesive cured with blue light. The device is placed first in HEPES buffered NaCl solution and then in $CaCl_2$ solution for five minutes to cross-link the alginate. Each implant is about 5 cm long, 1 mm in diameter, and contained approximately 2.5 million cells.

After the devices are filled and sealed, a silicone tether (Speciality Silcone Fabrication, Paso Robles, CA) (ID: 0.69, OD: 1.25) is then placed over the proximal end of the fiber. A radiopaque titanium plug is inserted in the lumen of the silicone tether to act as a radiographic marker. The devices are then placed in 100 mm tissue culture dishes in 1.5 ml PC-1 medium, and stored at 37°C, in a 5% $CO_2$ incubator for *in vitro* analysis and for storage until implantation.

The encapsulated cells are then implanted into the human sub-arachnoid space as follows:

**Surgical Procedure**

After establishing IV access and administering prophylactic antibiotics (cefazolin sodium, 1 gram IV), the patient is positioned on the operating table, generally in either the lateral decubitus or genu-pectoral position, with the lumbar spine flexed anteriorly. The operative field is sterily prepared and draped exposing the midline dorsal lumbar region from the levels of S-1 to L-1, and

allowing for intraoperative imaging of the lumbar spine with C-arm fluoroscopy. Local infiltration with 1.0% lidocaine is used to establish anesthesia of the skin as well as the periosteum and other deep connective tissue structures down to and including the ligamentum flavum.

A 3-5 cm skin incision is made in the parasagital plane 1-2 cm to the right or left of the midline and is continued down to the lumbodorsal fascia using electrocautery for hemostasis. Using traditional bony landmarks including the iliac crests and the lumbar spinous processes, as well as fluoroscopic guidance, and 18 gauge Touhy needle is introduced into the subarachnoid space between L-3 and L-4 via an oblique paramedian approach. The needle is directed so that it enters the space at a shallow, superiorly directed angle that is no greater than 30-35° with respect to the spinal cord in either the sagittal or transverse plane. Appropriate position of the tip of the needle is confirmed by withdrawal of several ml of cerebrospinal fluid (CSF) for preimplantation catecholamine, enkephalin, glucose, and protein levels and cell counts.

The Touhy needle hub is reexamined to confirm that the opening at the tip is oriented superiorly (opening direction is marked by the indexing notch for the obturator on the needle hub), and the guide wire is passed down the lumen of the needle until it extends 4-5 cm into the subarachnoid space (determined by premeasuring). Care is taken during passage of the wire that there is not resistance to advancement of the wire out of the needle and that the patient does not

complain of significant neurogenic symptoms, either of which observations might indicate misdirection of the guide wire and possible impending nerve root or spinal cord injury.

After the guide wire appears to be appropriately placed in the subarachnoid space, the Touhy needle is separately withdrawn and removed from the wire. The position of the wire in the midline of the spinal canal, anterior to the expected location of the caud equina, and without kinks or unexplainable bends is then confirmed with fluoroscopy. After removal of the Touhy needle the guide wire should be able to be moved freely into and out of the space with only very slight resistance due to the rough surface of the wire running through the dense and fibrous ligamentum flavum.

The 7 French dilator is then placed over the guide wire and the wire is used to direct the dilator as it is gently but firmly pushed through the fascia, paraspinous muscle, and ligamentum flavum, following the track of the wire toward the subarachnoid space. Advancement of the 7 French dilator is stopped and the dilator removed from the wire as soon as a loss of resistance is detected after passing the ligamentum flavum. This is done in order to avoid advancing and manipulating this relatively rigid dilator within the subarachnoid space to any significant degree.

After the wire track is "overdilated" by the 7 French dilator, the 6 French dilator and cannula sheath are assembled and placed over the guide wire. The 6 French dilator and cannula are advanced carefully into the subarachnoid space until the opening tip of

the cannula is positioned 7 cm within the space. As with the 7 French dilator, the assembled 6 French dilator and cannula are directed by the wire within the lumen of the dilator. Position within the subarachnoid space is determined by premeasuring the device and is grossly confirmed by fluoroscopy. Great care is taken with manipulation of the dilators and cannula within the subarachnoid space to avoid misdirection and possible neurologic injury.

When appropriate positioning of the cannula is assured, the guide wire and the 6 French dilator are gently removed from the lumen of the cannula in sequence. Depending on the patient's position on the operating table, CSF flow through the cannula at this point should be noticeable and may be very brisk, requiring capping the cannula or very prompt placement of the capsule implant in order to prevent excessive CSF.

The encapsulated (transformed cells) is provided in a sterile, double envelope container, bathed in transport medium, and fully assembled including a tubular silicone tether. Prior to implantation through the cannula and into the subarachnoid space, the capsule is transferred to the insertion kit tray where it is positioned in a location that allowed the capsule to be maintained in transport medium while it is grossly examined for damage or major defects, and while the silicone tether is trimmed, adjusting its length to the pusher and removing the hemaclip™ that plugs its external end.

The tether portion of the capsule is mounted onto the stainless steel pusher by inserting the small

diameter wire portion of the pusher as the membrane portion of the device is carefully introduced into the cannula. The capsule is advanced until the tip of the membrane reaches a point that is 2-10 mm within the cranial tip of the cannula in the subarachnoid space. This placement is achieved by premeasuring the cannula and the capsule-tether-pusher assembly, and it assures that the membrane portion of the capsule is protected by the cannula for the entire time that it is being advanced into position.

After the capsule is positioned within the cannula, the pusher is used to hold the capsule in position (without advancing or withdrawing) in the subarachnoid space while the cannula is completely withdrawn from over the capsule and pusher. The pusher is then removed from the capsule by sliding its wire portion out of the silicone tether. Using this method the final placement of the capsule is such that the 5 cm long membrane portion of the device lay entirely within the CSF containing subarachnoid space ventral to the cauda equina. It is anchored at its caudal end by a roughly 1-2 cm length of silicone tether that runs within the subarachnoid space before the tether exits through the dura and ligamentum flavum. The tether continues externally from this level through the paraspinous muscle and emerges from the lumbodorsal fascia leaving generally 10-12 cm of free tether material that is available for securing the device.

CSF leakage is minimized by injecting fibrin glue (Tissel®) into the track occupied by the tether in the paraspinous muscle, and by firmly closing the superficial fascial opening of the track with a purse-

string suture. The free end of the tether is then anchored with non-absorbable suture and completely covered with a 2 layer closure of the skin and subcutaneous tissue.

The patient is then transferred to the neurosurgical recovery area and kept at strict bed rest, recumbent, for 24 hours postoperatively. Antibiotic prophylaxis is also continued for 24 hours following the implantation procedure.

**Sequences**

The following is a summary of the sequences set forth in the Sequence Listing:

SEQ ID NO:1 -- DNA sequence of oligo oCNTF-003
SEQ ID NO:2 -- DNA sequence of oligo oIgSP-018
SEQ ID NO:3 -- DNA sequence of IgSP-hPOMC fusion
SEQ ID NO:4 -- DNA sequence of IgSP-hPOMC-ΔACTH fusion
SEQ ID NO:5 -- DNA sequence of oligo orTH-052
SEQ ID NO:6 -- DNA sequence of oligo orTH-053
SEQ ID NO:7 -- DNA sequence of oligo orTH-054
SEQ ID NO:8 -- DNA sequence of oligo orTH-078
SEQ ID NO:9 -- DNA sequence of oligo oIRES-057
SEQ ID NO:10 -- DNA sequence of oligo obDBH-065
SEQ ID NO:11 -- DNA sequence of oligo oIRES-bDBH-064
SEQ ID NO:12 -- DNA sequence of oligo oIRES-bDBH-066
SEQ ID NO:13 -- DNA sequence of oligo oIRE-068
SEQ ID NO:14 -- DNA sequence of oligo orTHΔ-073
SEQ ID NO:15 -- DNA sequence of oligo ohPOMC-IRES-069
SEQ ID NO:16 -- DNA sequence of rTHΔ1-155
SEQ ID NO:17 -- DNA sequence of rTHΔ+KS
SEQ ID NO:18 -- DNA sequence of rTHΔ-IRES-bDBH
SEQ ID NO:19 -- DNA sequence of rTHΔKS-IRES-bDBH

SEQ ID NO:20 -- DNA sequence of oligo ohPOMC-IRES-070
SEQ ID NO:21 -- DNA sequence of oligo oIRES-rTHΔ-071
SEQ ID NO:22 -- DNA sequence of oligo orIRES-rTHΔ-072
SEQ ID NO:23 -- DNA sequence of IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH-068 fusion
SEQ ID NO:24 -- DNA sequence oIRES-074
SEQ ID NO:25 -- DNA sequence of oligo oZeocin-077
SEQ ID NO:26 -- DNA sequence of oligo oIRES-Zeocin-075
SEQ ID NO:27 -- DNA sequence of oligo oIRES-Zeocin-076
SEQ ID NO:28 -- DNA sequence IgSP-hPOMCΔACTH-IRES-rTHΔ-IRES-bDBH-IRES-Zeocin-073
SEQ ID NO:29 -- DNA sequence of proA+KS
SEQ ID NO:30 -- DNA sequence of IRES fragment

**Deposits**

RINa/ProA/POMC/TH-IRES-DBH cells, transformed to produce a catecholamine, an enkephalin and an endorphin, as described above in the example (and in Table 2), named RINa/ProA/P030/P088, have been deposited. The deposit was made in accordance with the Budapest Treaty and was deposited at the American Type Culture Collection, Rockville, Maryland, U.S.A. on June 7, 1995. The deposit received accession number CRL 11921.

The foregoing description has been for the purpose of illustration and description only. This description is not intended to limit the invention to the precise form exemplified. It is intended that the scope of the invention be defined by the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CytoTherapeutics, Inc. (For purposes of all designated states except US)
Shou Wong (For purposes of US only)
Joel Saydoff (For purposes of US only)

(ii) TITLE OF INVENTION: PAIN CELL LINE

(iii) NUMBER OF SEQUENCES: 30

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/481,917
(B) FILING DATE: 07-JUNE-1995

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(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: cCNTF-003

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCCGGATCCG CGTCACCCCT AGAGTCGAGC TGT 33

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIgSP-018

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTTCCCGGGA AAGCCGAATT CAC 23

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 849 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: IgSP-hPOMC

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..43

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 44..89

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 90..168

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 807..849

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 43..186
(D) OTHER INFORMATION: /product= "IgSp region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 187..806
(D) OTHER INFORMATION: /product= "hPOMC region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGATCCGCGT CACCCCTAGA GTCGAGCTGT GACGGTCCTT ACAATGAAAT GCAGCTGGGT 60

TATCTTCTTC CTGATGGCAG TGGTTACAGG TAAGGGGCTC CCAAGTCCCA AACTTGAGGG 120

TCCATAAACT CTGTGACAGT GGCAATCACT TTGCCTTTCT TTCTACAGGG GTGAATTCGG 180

CTTTCCCGGG AAATGGCGAC GAGCAGCCTC TGACCGAGAA CCCCCGGAAG TACGTCATGG 240

GCCACTTCCG CTGGGACCGA TTCGGCCGCC GCAACAGCAG CAGCAGCGGC AGCAGCGGCG 300

CAGGGCAGAA GCGCGAGGAC GTCTCAGCGG GCGAAGACTG CGGCCCGCTG CCTGAGGGCG 360

GCCCCGAGCC CCGCAGCGAT GGTGCCAAGC CGGGCCCCGG CGAGGGCAAG CGCTCCTACT 420

CCATGGAGCA CTTCCGCTGG GGCAAGCCCG TGGGCAAGAA GCGCGCCCCA GTGAAGGTGT 480

ACCCTAACGG CGCCGAGGAC GAGTCCGGCG AGGCCTTCCC CCTGGAGTTC AAGAGGGAGC 540

TGACTGGCCA GCGACTCCGG GAGGGAGATG GCCCCGACGG CCCTGCCGAT GACGGCGCAG 600

GGCCCCAGGC CGACCTGGAG CACAGCCTGC TGGTGGCCGC CGAGAAGAAG GACGAGGGCC 660

CCTACAGGAT GGAGCACTTC GGCTGGGGCA GCCCGCCCAA GGACAAGCGC TACGGCGGTT 720

TCATGACCTC CGAGAAGAGC CAGACCGCCC TGGTGACGCT GTTCAAAAAC GCCATCATCA 780

AGAACGCCTA CAAGAAGGGC GAGTGAGGGC ACAGCGGGCC CCAGGGCTAC CCTCCCCCAG 840

GAGGTCGAC 849

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 525 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: IgSP-hPOMCDACTH

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..43

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 44..89

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 90..168

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 169..482

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 483..525

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 44..188
(D) OTHER INFORMATION: /product= "IgSP region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 189..482
(D) OTHER INFORMATION: /product= "hPOMC region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GGATCCGCGT CACCCCTAGA GTCGAGCTGT GACGGTCCTT ACAATGAAAT GCAGCTGGGT 60

TATCTTCTTC CTGATGGCAG TGGTTACAGG TAAGGGGCTC CCAAGTCCCA AACTTGAGGG 120

TCCATAAACT CTGTGACAGT GGCAATCACT TTGCCTTTCT TTCTACAGGG GTGAATTCGG 180

CTTTCCCGGC CTTCCCCCTG GAGTTCAAGA GGGAGCTGAC TGGCCAGCGA CTCCGGGAGG 240

GAGATGGCCC CGACGGCCCT GCCGATGACG GCGCAGGGGC CCAGGCCGAC CTGGAGCACA 300

GCCTGCTGGT GGCGGCCGAG AAGAAGGACG AGGGCCCCTA CAGGATGGAG CACTTCCGCT 360

GGGGCAGCCC GCCCAAGGAC AAGCGCTACG CCGGTTTCAT GACCTCCGAG AAGAGCCAGA 420

CGCCCCTGGT GACGCTGTTC AAAAACGCCA TCATCAAGAA CGCCTACAAG AAGGGCGAGT 480

GAGGGCACAG CGGGCCCCAG GGCTACCCTC CCCCAGGAGG TCGAC 525

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: orTH-052

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCCAAGCTTG CACTATGCCC ACCCCCAGCG 30

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: orTH-053

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCCGGATCCT ATGCATTTAG CTAATGGCAC 30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: orTH-054

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCCAAGCTTA TGGTCCCCTG GTTCCCAAGA 30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: orTH-078

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCCAAGCTTC GCCACCATGG TCCCCTGGTT CCC 33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIRES-057

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AAAGGATCCG CCCCTCTCCC TCCCCCCCCC 30

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: cbDBH-065

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AAAGCGGCCG CCCAGGTTCA GCCTTTGCCC 30

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIRES-bDBH-064

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTTGCCACAA CCATGTACGG CACCGCGGTG 30

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIRES-bDBH-066

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGCGGTGCCG TACATGGTTG TGGCAAGCTT 30

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIgSP-068

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAAGATATCG CGGCGGGTC ACCCCTAGAG 30

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: orTHD-073

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATACACCTGG TCAGAGAAGC CGGGG 25

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: chPOMC-IRES-069

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGGGGAGGG AGAGGGGGCC GCTGTGCCCT

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1030 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: rTHD

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..6

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 7..1017

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 1018..1030

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AAGCTTATGG TCCCCTGGTT CCCAAGAAAA GTGTCGGAAT TGGACAAGTG TCACCACCTG 60

GTCACCAAGT TTGACCCTGA TCTGGACCTG GACCACCCGG GCTTCTCTGA CCAGGTGTAT 120

CGCCAGCGTC GGAAGCTGAT TGCAGAGATT GCCTTCCAGT ACAAGCACGG TGAACCAATT 180

CCCCATGTGG AATACACAGC GGAAGAGATT GCTACCTGGA AGGAGGTATA TGTCACGCTG 240

AAGGGCCTCT ATGCTACCCA TGCCTGCCGG GAGCACCTGG AGGGTTTCCA GCTTCTGGAA 300

CGGTACTGTG GCTACCGAGA GGACAGCATC CCACAGCTGG AGGACGTGTC CCGCTTCTTG 360

AAGGAGCGGA CTGGCTTCCA GCTGCGACCC GTGGCCGGTC TACTGTCCGC CCGTGATTTT 420

CTGGCCAGTC TGGCCTTCCG CGTGTTTCAA TGCACCCAGT ATATCCGCCA TGCCTCCTCA 480

CCTATGCATT CACCTGAGCC GGACTGCCTC CATGAGCTGT TGGGACATGT ACCCATGTTG 540

GCTGACCGCA CATTTGCCCA GTTCTCCCAG GACATTGGAC TTGCATCTCT GGGGGCCTCA 600

GATGAAGAAA TTGAAAAACT CTCCACGGTG TACTGGTTCA CTGTGGAATT CGGGCTATGT 660

AAACAGAATG GGGAGCTGAA GGCTTATGGT GCAGGGCTGC TGTCTTCCTA CGGAGAGCTC 720

CTGCACTCCC TGTCAGAGGA GCCTGAGGTC CGAGCCTTTG ACCCAGACAC AGCAGCTGTG 780

CAGCCCTACC AAGATCAAAC CTACCAGCCT GTGTACTTTG TGTCCGAGAG CTTCAATGAC 840

GCCAAGGACA AGCTCAGGAA CTATGCCTCT CGTATCCAGC GCCCATTCTC TGTGAAGTTT 900

GACCCGTACA CACTGGCCAT TGACGTACTG GACAGCCCTC ACACCATCCA GCGCTCCTTG 960

GAGGGGGTCC AGGATGAGCT GCACACCCTG GCCCAGGCAC TGAGTGCCAT TAGCTAAATG 1020

CATAGGATCC 1030

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1037 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: rTHIKS

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..13

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 14..1024

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 1025..1037

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AAGCTTCGCC ACCATGGTCC CCTGGTTCCC AAGAAAAGTG TCGGAATTGG ACAAGTGTCA 60

CCACCTGGTC ACCAAGTTTG ACCCTGATCT GGACCTGGAC CACCCGGCCT TCTCTGACCA 120

GGTGTATCGC CAGCGTCGGA AGCTGATTGC AGAGATTGCC TTCCAGTACA AGCACGGTGA 180

ACCAATTCCC CATGTGGAAT ACACAGCGGA AGAGATTGCT ACCTGGAAGG AGGTATATGT 240

CACGCTGAAG GGCCTCTATG CTACCCATGC CTGCCGGGAG CACCTGGAGG GTTTCCAGCT 300

TCTGGAACGG TACTGTGGCT ACCGAGAGGA CAGCATCCCA CAGCTGGAGG ACGTGTCCCG 360

CTTCTTGAAG GAGCGGACTG GCTTCCAGCT GCGACCCGTG GCCGGTCTAC TGTCCGCCCG 420

TGATTTTCTG GCCAGTCTGG CCTTCCGCGT GTTTCAATGC ACCCAGTATA TCCGCCATGC 480

CTCCTCACCT ATGCATTCAC CTGAGCCGGA CTGCTGCCAT GACCTGTTGG GACATGTACC 540

CATGTTGGCT GACCGCACAT TTGCCCAGTT CTCCCAGGAC ATTGGACTTG CATCTCTGGG 600

GGCCTCAGAT GAAGAAATTG AAAAACTCTC CACGGTGTAC TGGTTCACTG TGGAATTCGG 660

GCTATGTAAA CAGAATGGGG AGCTGAAGGC TTATGGTGCA GGGCTGCTGT CTTCCTACGG 720

AGAGCTCCTG CACTCCCTGT CAGAGGAGCC TGAGGTCCGA GCCTTTGACC CAGACACAGC 780

AGCTGTGCAG CCCTACCAAG ATCAAACCTA CCAGCCTGTG TACTTTGTGT CCGAGAGCTT 840

CAATGACGCC AAGGACAAGC TCAGGAACTA TGCCTCTCGT ATCCAGCGCC CATTCTCTGT 900

GAAGTTTGAC CCGTACACAC TGGCCATTGA CGTACTGGAC AGCCCTCACA CCATCCAGCG 960

CTCCTTGGAG GGGGTCCAGG ATGAGCTGCA CACCCTGGCC CACGCACTGA GTGCCATTAG 1020

CTAAATGCAT AGGATCC 1037

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3425 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: rTH-IRES-bDBH

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..6

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 7..1017

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1018..1617

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1618..3411

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 3412..3425

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1025..1617
(D) OTHER INFORMATION: /product= "IRES sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AAGCTTATGG TCCCCTGGTT CCCAAGAAAA GTGTCGGAAT TGGACAAGTG TCACCACCTG 60

GTCACCAAGT TTGACCCTGA TCTGGACCTG GACCACCCGG GCTTCTCTGA CCAGGTGTAT 120

CGCCAGCGTC GGAAGCTGAT TGCAGAGATT GCCTTCCAGT ACAAGCACGG TGAACCAATT 180

CCCCATGTGG AATACACAGC GGAAGAGATT GCTACCTGGA AGGAGGTATA TGTCACGCTG 240

AAGGGCCTCT ATGCTACCCA TGCCTGCCGG GAGCACCTGG AGGGTTTCCA GCTTCTGGAA 300

CGGTACTGTG GCTACCGAGA GGACAGCATC CCACAGCTGG AGGACGTGTC CCGCTTCTTG 360

AAGGAGCGGA CTGGCTTCCA GCTGGACCC GTGGCCGGTC TACTGTCCGC CCGTGATTTT 420

CTGGCCAGTC TGGCCTTCCG CGTGTTTCAA TGCACCCAGT ATATCCGCCA TGCCTCCTCA 480

CCTATGCATT CACCTGAGCC GGACTGCTGC CATGAGCTGT TGGGACATGT ACCCATGTTG 540

GCTGACCGCA CATTTGCCCA GTTCTCCCAG GACATTGGAC TTGCATCTCT GGGGGCCTCA 600

GATGAAGAAA TTGAAAAACT CTCCACGGTG TACTGGTTCA CTGTGGAATT CGGGCTATGT 660

AAACAGAATG GGGAGCTGAA GGCTTATGGT GCAGGCTGC TGTCTTCCTA CGGAGAGCTC 720

CTGCACTCCC TGTCAGAGGA GCCTGAGGTC CGAGCCTTTG ACCCAGACAC AGCAGCTGTG 780

CAGCCCTACC AAGATCAAAC CTACCAGCCT GTGTACTTTG TGTCCGAGAG CTTCAATGAC 840

GCCAAGGACA AGCTCAGGAA CTATGCCTCT CGTATCCAGC GCCCATTCTC TGTGAAGTTT 900

GACCCGTACA CACTGGCCAT TGACGTACTG GACAGCCCTC ACACCATCCA GGGCTCCTTG 960

GAGGGGGTCC AGGATGAGCT GCACACCCTG GCCCACGCAC TGAGTGCCAT TAGCTAAATG 1020

CATAGGATCC GCCCCTCTCC CTCCCCCCCC CCTAACGTTA CTGGCCGAAG CCGCTTGGAA 1080

TAAGGCCGGT GTGCGTTTGT CTATATGTTA TTTTCCACCA TATTGCCGTC TTTTGGCAAT 1140

GTGAGGGCCC GGAAACCTGG CCCTGTCTTC TTGACGAGCA TTCCTAGGGG TCTTTCCCCT 1200

CTCGCCAAAG GAATGCAAGG TCTGTTGAAT GTCGTGAAGG AAGCAGTTCC TCTGGAAGCT 1260

TCTTGAAGAC AAACAACGTC TGTAGCGACC CTTTGCAGGC AGCGGAACCC CCCACCTGGC 1320

GACAGGTGCC TCTGCGGCCA AAAGCCACGT GTATAAGATA CACCTGCAAA GGCGGCACAA 1380

CCCCAGTGCC ACGTTGTGAG TTGGATAGTT GTGGAAAGAG TCAAATGGCT CTCCTCAAGC 1440

GTATTCAACA AGGGGCTGAA GGATGCCCAG AAGGTACCCC ATTGTATGGG ATCTGATCTG 1500

GGGCCTCGGT GCACATGCTT TACATGTGTT TAGTCGAGGT TAAAAAACGT CTAGGCCCCC 1560

CGAACCACGG GGACGTGGTT TTCCTTTGAA AAACACGATG ATAAGCTTGC CACAACCATG 1620

TACGGCACCG CGGTGGCGGT CTTCCTGGTC ATCCTCGTGG CTGCACTGCA GGGCTCGGCT 1680

CCCGCCGAGA GCCCCTTCCC CTTCCACATC CCCCTGGACC CCGAGGGGAC CCTGGAGCTG 1740

TCCTGGAACA TCAGCTATGC GCAGGAGACC ATCTACTTCC AGCTCCTGGT GCGGGAGCTC 1800

AAGGCTGGTG TCCTGTTTGG GATGTCGGAC CGAGGGGAGC TGGAGAATGC TGACTTGGTG 1860

GTGCCTCTGGA CTGACAGGGA CGGCGCCTAC TTTGGGGATG CCTGGAGTGA CCAGAAGGGG 1920

CAGGTCCACC TGGACTCCCA GCAGGATTAC CAGCTTCTGC GGGCACAGAG GACTCCAGAA 1980

GGCCTGTACC TGCTCTTCAA GAGGCCTTTT GGCACCTGTG ACCCCAACGA CTACCTCATC 2040

GAGGACGGCA CCGTCCACCT GGTGTATGGA TTCCTGGAGG AGCCGCTCTG GTCGCTGGAG 2100

TCCATCAACA CATCCGGCTT GCACACGGGG CTGCAGAGGG TGCAGCTGCT GAAGCCCAGC 2160

ATCCCCAAGC CGGCCCTGCC CGCGGACACG CGCACCATGG AGATCCGCGC CCCCGACGTC 2220

CTCATCCCCG GCCAGCAGAC CACGTACTGG TGCTACGTGA CCGAGCTCCC GGACGGCTTC 2280

CCCCGGCACC ACATCGTCAT GTACGAGCCC ATCGTCACCG AGGGCAACGA GGCGCTGGTG 2340

CACCACATGG AGGTCTTCCA GTCGCCCGCC GAGTTCGAGA CCATCCCCCA CTTCAGCGGG 2400

CCCTGCGACT CCAAGATGAA GCCGCAGCGG CTCAACTTCT GCCGTCACGT GCTGGCCGCC 2460

TGGGCCCTGG GCGCCAAGCC CTTTTACTAC CCAGAGGAAG CAGCCCTGGC CTTCGGGGGG 2520

CCCGGCTCCT CCAGATTTCT CCGCCTGGAA GTTCACTACC ACAACCCACT GGTGATAACA 2580

GCCCGCGCGG ACTCCTCGGG CATCCGCCTG TACTACACGG CTGCGCTGCG GCGCTTCGAC 2640

GCGGGCATCA TGGAGCTGGG CCTGGCGTAC ACGCCCGTGA TGGCCATCCC CCCGCAGGAG 2700

ACGGCCTTCG TCCTCACCGG CTACTGCACG GACAAGTGCA CCCAGCTGGC CCTGCCCGCC 2760

TCAGGGATTC ACATCTTCGC CTCTCAGCTC CACACGCACC TGACCGGCCG GAAGGTGGTC 2820

ACAGTGCTGG CCAGGGACGG CGGGGAGACA GAGATCGTGA ACAGGGACAA CCACTACAGC 2880

CCACACTTCC AGGAGATCCG CATGTTGAAG AAGGTCGTGT CTGTCCAGCC GGGAGACGTG 2940

CTCATCACCT CTTGCACATA CAACACGGAA GACAGGAGGC TGGCCACCGT GGGGGGCTTC 3000

GGGATCCTGG AGGAGATGTG CGTCAACTAT GTGCACTACT ACCCCCAGAC GCAGCTGGAG 3060

CTCTGCAAGA GCGCCGTGGA CCCTGGCTTC CTGCACAAGT ACTTCCGCCT CGTGAACAGG 3120

TTCAACAGCG AGGAAGTCTG CACCTGCCCC CAGGCGTCTG TCCCTGAGCA GTTTGCCTCC 3180

GTGCCCTGGA ACTCCTTCAA CCGCGAGGTG CTCAAGGCCC TGTACGGCTT CGCACCCATC 3240

TCCATGCACT GCAACAGGTC CTCGCCCGTC CGCTTCCAGG CGGAGTGGAA TCGGCAGCCC 3300

CTGCCTGAGA TCGTGTCCAG GTTGGAAGAG CCCACCCCTC ACTGCCCAGC CAGCCAGGCT 3360

CAGAGCCCCG CCGGCCCCAC CGTGCTGAAC ATCAGTGGGG GCAAAGGCTG AACGTGGGCG 3420

GCCGC 3425

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3432 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: rTHDKS-IRES-bDBH

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..13

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 14..1024

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1025..1624

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1625..3418

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 3419..3432

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1032..1624
(D) OTHER INFORMATION: /product= "IRES sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AAGCTTCGCC ACCATGGTCC CCTGGTTCCC AAGAAAAGTG TCGGAATTGG ACAAGTGTCA 60

CCACCTGGTC ACCAAGTTTG ACCCTGATCT GGACCTGGAC CACCCGGGCT TCTCTGACCA 120

GGTGTATCGC CAGCGTCGGA AGCTGATTGC AGAGATTGCC TTCCAGTACA AGCACGGTGA 180

ACCAATTCCC CATGTGGAAT ACACAGCGGA AGAGATTGCT ACCTGGAAGG AGGTATATGT 240

CACGCTGAAG GGCCTCTATG CTACCCATGC CTGCCGGGAG CACCTGGAGG GTTTCCAGCT 300

TCTGGAACGG TACTGTGGCT ACCGAGAGGA CAGCATCCCA CAGCTGGAGG ACGTGTCCCG 360

CTTCTTGAAG GAGCGGACTG GCTTCCAGCT GCGACCCGTG GCCGGTCTAC TGTCCGCCCG 420

TGATTTTCTG GCCAGTCTGG CCTTCCGCGT GTTTCAATGC ACCCAGTATA TCCGCCATGC 480

CTCCTCACCT ATGCATTCAC CTGAGCCGGA CTGCTGCCAT GAGCTGTTGG GACATGTACC 540

CATGTTGGCT GACCGCACAT TTGCCCAGTT CTCCCAGGAC ATTGGACTTG CATCTCTGGG 600

GGCCTCAGAT GAAGAAATTG AAAAACTCTC CACGGTGTAC TGGTTCACTG TGGAATTCGG 660

GCTATGTAAA CAGAATGGGG AGCTGAAGGC TTATGGTGCA GGGCTGCTGT CTTCCTACGG 720

AGAGCTCCTG CACTCCCTGT CAGAGGAGCC TGAGGTCCGA GCCTTTGACC CAGACACAGC 780

AGCTGTGCAG CCCTACCAAG ATCAAACCTA CCAGCCTGTG TACTTTGTGT CCGAGAGCTT 840

CAATGACGCC AAGGACAAGC TCAGGAACTA TGCCTCTCGT ATCCAGCGCC CATTCTCTGT 900

GAAGTTTGAC CCGTACACAC TGGCCATTGA CGTACTGGAC AGCCCTCACA CCATCCAGCG 960

CTCCTTGGAG GGGGTCCAGG ATGAGCTGCA CACCCTGGCC CACGCACTGA GTGCCATTAG 1020

CTAAATGCAT AGGATCCGCC CCTCTCCCTC CCCCCCCCCT AACGTTACTG GCCGAAGCCG 1080

CTTGGAATAA GGCCGGTGTG CGTTTGTCTA TATGTTATTT TCCACCATAT TGCCGTCTTT 1140

TGGCAATGTG AGGGCCCGGA AACCTGGCCC TGTCTTCTTG ACGAGCATTC CTAGGGGTCT 1200

TTCCCCTCTC GCCAAAGGAA TGCAAGGTCT GTTGAATGTC GTGAAGGAAG CAGTTCCTCT 1260

GGAAGCTTCT TGAAGACAAA CAACGTCTGT AGCGACCCTT TGCAGGCAGC GGAACCCCCC 1320

ACCTGGCGAC AGGTGCCTCT GCGGCCAAAA GCCACGTGTA TAAGATACAC CTGCAAAGGC 1380

GGCACAACCC CAGTGCCACG TTGTGAGTTG GATAGTTGTG GAAAGAGTCA AATGGCTCTC 1440

CTCAAGCGTA TTCAACAAGG GGCTGAAGGA TGCCCAGAAG GTACCCCATT GTATGGGATC 1500

TGATCTGGGG CCTCGGTGCA CATGCTTTAC ATGTGTTTAG TCGAGGTTAA AAAACGTCTA 1560

GGCCCCCCGA ACCACGGGGA CGTGGTTTTC CTTTGAAAAA CACGATGATA AGCTTGCCAC 1620

AACCATGTAC GGCACCGCGG TGGCCGTCTT CCTGGTCATC CTCGTGGCTG CACTGCAGGG 1680

CTCGGCTCCC GCCGAGAGCC CCTTCCCCTT CCACATCCCC CTGGACCCCG AGGGGACCCT 1740

GGAGCTGTCC TGGAACATCA GCTATGCGCA GGAGACCATC TACTTCCAGC TCCTGGTGCG 1800

GGAGCTCAAG GCTGGTGTCC TGTTTGGGAT GTCGGACCGA GGGGAGCTGG AGAATGCTGA 1860

CTTGGTGGTG CTCTGGACTG ACAGGGACGG CGCCTACTTT GGGGATGCCT GGAGTGACCA 1920

GAAGGGCAG GTCCACCTGG ACTCCCAGCA GGATTACCAG CTTCTGCGGG CACAGAGGAC 1980

TCCAGAAGGC CTGTACCTGC TCTTCAAGAG GCCTTTTGGC ACCTGTGACC CCAACGACTA 2040

CCTCATCGAG GACGGCACCG TCCACCTGGT GTATGGATTC CTGGAGGAGC CGCTCCGGTC 2100

GCTGGAGTCC ATCAACACAT CCGGCTTGCA CACGGGGCTG CAGAGGGTGC AGCTGCTGAA 2160

GCCCAGCATC CCCAAGCCGG CCCTGCCCGC GGACACGGGC ACCATGGAGA TCCGCGCCCC 2220

CGACGTCCTC ATCCCCGGCC AGCAGACCAC GTACCTGGTG TACGTGACCG AGCTCCCCGA 2280

CGGCTTCCCC CGGCACCACA TCGTCATGTA CGAGCCCATC GTCACCGAGG GCAACGAGGC 2340

GCTGGTGCAC CACATGGAGG TCTTCCAGTG CGCCGCCGAG TTCGAGACCA TCCCCCACTT 2400

CAGCGGGCCC TGCGACTCCA AGATGAAGCC GCAGCGGCTC AACTTCTGCC GTCACGTGCT 2460

GGCCGCCTGG GCCCTGGGCG CCAAGGCCTT TTACTACCCA GAGGAAGCAG GCCTGGCCTT 2520

CGGCGGCCCC GGCTCCTCCA GATTTCTCCG CCTGGAAGTT CACTACCACA ACCCACTGGT 2580

GATAACAGGC CGGCGCGACT CCTCGGGCAT CGGCCTGTAC TACACGGCTG CGCTGCGGCG 2640

CTTCGACCGG GGCATCATGG AGCTGGGCCT GGCGTACCAG CCCGTGATGG CCATCCCCCC 2700

GCAGGAGACG GCCTTCGTCC TCACCGGCTA CTGCACGGAC AAGTGCACCC AGCTGGCCCT 2760

GCCCGCCTCA GGGATTCACA TCTTCGCCTC TCAGCTCCAC ACGCACCTGA CGGCCCGAA 2820

GGTGGTCACA GTGCCTGGCA GGGACGGCCG GGAGACAGAG ATCGTGAACA GGGACAACCA 2880

CTACAGCCCA CACTTCCAGG AGATCCGCAT GTTGAAGAAG GTCGTGTCTG TCCAGCCGGG 2940

AGACGTGCTC ATCACCTCTT GCACATACAA CACGGAAGAC AGGAGGCTGG CCACCGTGGG 3000

GGGCTTCGGG ATCCTGGAGG AGATGTGCGT CAACTATGTG CACTACTACC CCCAGACGCA 3060

GCTGGAGCTC TGCAAGAGCG CCGTGGACCC TGGCTTCCTG CACAAGTACT TCCGCCTCGT 3120

GAACAGGTTC AACAGCGAGG AAGTCTGCAC CTGCCCCCAG GCGTCTGTCC CTGAGCAGTT 3180

TGCCTCCGTG CCCTGGAACT CCTTCAACCG CGAGGTGCTC AAGCCCCTGT ACGGCTTCGC 3240

ACCCATCTCC ATGCACTGCA ACAGGTCCTC GGCCGTCCGC TTCCAGGGCG AGTGGAATCG 3300

GCAGCCCCTG CCTGAGATCG TGTCCAGGTT CGAAGAGCCC ACCCCTCACT GCCCAGCCAG 3360

CCAGGCTCAG AGCCCCGCCG GCCCCACCGT GCTGAACATC AGTGGGGGCA AAGGCTGAAC 3420

GTGGGCGGCC GC 3432

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: chPOMC-IRES-070

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AGGGCACAGC GGGCCCCTCT CCCTCCCCCC 30

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIRES-rTHD-071

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GAACCAGGGG ACCATGGTTG TGGCAAGCTT 30

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIRES-rTHD-072

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTTGCCACAA CCATGGTCCC CTGGTTCCCA 30

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4499 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: pamc-th-dbh fusion

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..43

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 44..89

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 90..168

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 169..482

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 483..1080

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1081..2091

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 2092..2691

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 2692..4485

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 4486..4499

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCGCCCCGGT CACCCCTAGA GTCGAGCCTGT GACGGTCCTT ACAATGAAAT GCAGCTGGGT 60

TATCTTCTTC CTGATGGCAG TGGTTACAGG TAAGGGGCTC CCAAGTCCCA AACTTGAGGG 120

TCCATAAACT CTGTTGACAGT GGCAATCACT TTGCCTTTCT TTCTACAGGG GTGAATTCGG 180

CTTTCCCGGC CTTCCCCCTG GAGTTCAAGA GGGAGCTGAC TGGCCAGCGA CTCCGGGAGG 240

GAGATGGCCC CGACGGCCCT GCCGATGACG GCGCAGGGGC CCAGGCCGAC CTGGAGCACA 300

GCCTGCTGGT GGCGCCCGAG AAGAAGGACG AGGGCCCCTA CAGGATGGAG CACTTCCGCT 360

GGGGCAGCCC GCCCAAGGAC AAGCGCTACG GCGGTTTCAT GACCTCCGAG AAGAGCCAGA 420

CGCCCCTGGT GACGCTGTTC AAAAACGCCA TCATCAAGAA CGCCTACAAG AAGGGCGAGT 480

GAGGGCACAG CGGGCCCCTC TCCCTCCCCC CCCCCTAACG TTACTGGCCG AAGCCGCTTG 540

GAATAAGGCC GGTGTGCGTT TGTCTATATG TTATTTTCCA CCATATTGCC GTCTTTTGGC 600

AATGTGAGGG CCCCGAAACC TGGCCCTGTC TTCTTGACGA GCATTCCTAG GGGTCTTTCC 660

CCTCTCGCCA AAGGAATGCA AGGTCTGTTG AATGTCGTGA AGGAAGCAGT TCCTCTGGAA 720

GCTTCTTGAA GACAAACAAC GTCTGTAGCG ACCCTTTGCA GGCAGCGGAA CCCCCCACCT 780

GGCGACAGGT GCCTCTGCGG CCAAAAGCCA CGTGTATAAG ATACACCTGC AAAGGCGGCA 840

CAACCCCAGT GCCACGTTGT GAGTTGGATA GTTGTGGAAA GAGTCAAATG GCTCTCCTCA 900

AGCGTATTCA ACAAGGGGCT GAAGGATGCC CAGAAGGTAC CCCATTGTAT GGGATCTGAT 960

CTGGGGCCTC GGTGCACATG CTTTACATGT GTTTAGTCGA GGTTAAAAAA CGTCTAGGCC 1020

CCCCGAACCA CGGGGACGTG GTTTTCCTTT GAAAAACACG ATGATAAGCT TGCCACAACC 1080

ATGGTCCCCT GGTTCCCAAG AAAAGTGTCG GAATTGGACA AGTGTCACCA CCTGGTCACC 1140

AAGTTTGACC CTGATCTGGA CCTGGACCAC CCGGGCTTCT CTGACCAGGT GTATCGCCAG 1200

CGTCGGAAGC TGATTGCAGA GATTGCCTTC CAGTACAAGC ACGGTGAACC AATTCCCCAT 1260

GTGGAATACA CAGCGGAAGA GATTGCTACC TGGAAGGAGG TATATGTCAC GCTGAAGGGC 1320

CTCTATGCTA CCCATGCCTG CGGGGAGCAC CTGGAGGGTT TCCAGCTTCT GGAACGGTAC 1380

TGTGGCTACC GAGAGGACAG CATCCCACAG CTGGAGGACG TGTCCCGCTT CTTGAAGGAG 1440

CGGACTGGCT TCCAGCTGCG ACCCGTGGCC GGTCTACTGT CCGCCCGTGA TTTTCTGGCC 1500

AGTCTGGCCT TCCGCGTGTT TCAATGCACC CAGTATATCC GCCATGCCTC CTCACCTATG 1560

CATTCACCTG AGCCGGACTG CTGCCATGAG CTGTTGGGAC ATGTACCCAT GTTGGCTGAC 1620

CGCACATTTG CCCAGTTCTC CCAGGACATT GGACTTGCAT CTCTGGGGGC CTCAGATGAA 1680

GAAATTGAAA AACTCTCCAC GGTGTACTGG TTCACTGTGG AATTCGGGCT ATGTAAACAG 1740

AATGGGGAGC TGAAGGCTTA TGGTGCAGGG CTGCTGTCTT CCTACGGAGA GCTCCTGCAC 1800

TCCCTGTCAG AGGAGCCTGA GGTCCGAGCC TTTGACCCAG ACACAGCAGC TGTGCAGCCC 1860

TACCAAGATC AAACCTACCA GCCTGTGTAC TTTGTGTCCG AGAGCTTCAA TGACGCCAAG 1920

GACAAGCTCA GGAACTATGC CTCTCGTATC CAGCGCCCAT TCTCTGTGAA GTTTGACCCG 1980

TACACACTGG CCATTGACGT ACTGGACAGC CCTCACACCA TCCAGCGCTC CTTGGAGGGG 2040

GTCCAGGATG AGCTGCACAC CCTGGCCCAC GCACTGAGTG CCATTAGCTA AATGCATAGG 2100

ATCCGCCCCT CTCCCTCCCC CCCCCCTAAC GTTACTGGCC GAAGCCGCTT GGAATAAGGC 2160

CGGTGTGCGT TTGTCTATAT GTTATTTTCC ACCATATTGC CGTCTTTTGG CAATGTGAGG 2220

GCCCGAAAC CTGGCCCTGT CTTCTTGACG AGCATTCCTA GGGGTCTTTC CCCTCTCGCC 2280

AAAGGAATGC AAGGTCTGTT GAATGTCGTG AAGGAAGCAG TTCCTCTGGA AGCTTCTTGA 2340

AGACAAACAA CGTCTGTAGC GACCCTTTGC AGGCAGCGGA ACCCCCCACC TGGCGACAGG 2400

TGCCTCTGCG GCCAAAAGCC ACGTGTATAA GATACACCTG CAAAGGCGGC ACAACCCCAG 2460

TGCCACGTTG TGAGTTGGAT AGTTGTGGAA AGAGTCAAAT GGCTCTCCTC AAGCGTATTC 2520

AACAAGGGGC TGAAGGATGC CCAGAAGGTA CCCCATTGTA TGGGATCTGA TCTGGGGCCT 2580

CGGTGCACAT GCTTTACATG TGTTTAGTCG AGGTTAAAAA ACGTCTAGGC CCCCCGAACC 2640

ACGGGGACGT GGTTTTCCTT TGAAAAACAC GATGATAAGC TTGCCACAAC CATGTACGGC 2700

ACCGCGGTGG CGGTCTTCCT GGTCATCCTC GTGGCTGCAC TGCAGGGCTC GGCTCCCGCC 2760

GAGAGCCCCT TCCCCTTCCA CATCCCCCTG GACCCCGAGG GGACCCTGGA GCTGTCCTGG 2820

AACATCAGCT ATGGGCAGGA GACCATCTAC TTCCAGCTCC TGGTGGGGGA GCTCAAGGCT 2880

GGTGTCCTGT TTGGGATGTC GGACCGAGGG GAGCTGGAGA ATGCTGACTT GGTGGTGCTC 2940

TGGACTGACA GGGACGGGGC CTACTTTGGG GATGCCTGGA GTGACCAGAA GGGGCAGGTC 3000

CACCTGGACT CCCAGCAGGA TTACCAGCTT CTGCGGGCAC AGAGGACTCC AGAAGGCCTG 3060

TACCTGCTCT TCAAGAGGCC TTTTGGCACC TGTGACCCCA ACGACTACCT CATCGAGGAC 3120

GGCACCGTCC ACCTGGTGTA TGGATTCCTG GAGGAGCCGC TCCGGTCGCT GGAGTCCATC 3180

AACACATCCG GCTTGCACAC GGGGCTGCAG AGGGTGCAGC TGCTGAAGCC CAGCATCCCC 3240

AAGCCCGCCC TGCCCCGCGA CACGCGCACC ATGGAGATCC GCGCCCCCGA CGTCCTCATC 3300

CCCGGCCAGC AGACCACGTA CTGGTGCTAC GTGACCGAGC TCCCGGACGG CTTCCCCCCG 3360

CACCACATCG TCATGTACGA GCCCATCGTC ACCGAGGGCA ACGAGGCGCT GGTGCACCAC 3420

ATGGAGGTCT TCCAGTGCGC CGCCGAGTTC GAGACCATCC CCCACTTCAG CGGGCCCTGC 3480

GACTCCAAGA TGAAGCCGCA GCGGCTCAAC TTCTGCGGTC ACGTGCTGGC CGCCTGGGCC 3540

CTGGGCGCCA AGGCCTTTTA CTACCCAGAG GAAGCAGGCC TGGCCTTCGG GGGCGCCGGC 3600

TCCTCCAGAT TTCTCGGCCT GGAAGTTCAC TACCACAACC CACTGGTGAT AACAGGCGCG 3660

CGCGACTCCT CGGGCATCCG CCTGTACTAC ACGGCTGCGC TGCGGCGCTT CGACGCGGGC 3720

ATCATGGAGC TGGGCCTGGC GTACACGCCC GTGATGGCCA TCCCCCCGCA GGAGACGGCC 3780

TTCGTCCTCA CGGGCTACTG CACGGACAAG TGCACCCAGC TGGCCCTGCC CGCCTCAGGG 3840

ATTCACATCT TCGCCTCTCA GCTCCACACG CACCTGACCG GCGGAAGGT GGTCACAGTG 3900

CTGGCCAGGG ACGCGCGGGA GACAGAGATC GTGAACAGGG ACAACCACTA CAGCCCACAC 3960

TTCCAGGAGA TCGGCATGTT GAAGAAGGTC GTGTCTGTCC AGCCGGGAGA CGTGCTCATC 4020

ACCTCTTGCA CATACAACAC GGAAGACAGG AGGCTGGCCA CCGTGGGGGG CTTCGGGATC 4080

CTGGAGGAGA TGTCGGTCAA CTATGTGCAC TACTACCCCC AGACGCAGCT GGAGCTCTGC 4140

AAGAGCGCCG TGGACCCTGG CTTCCTGCAC AAGTACTTCC GCCTCGTGAA CAGGTTCAAC 4200

AGGAGGAAG TCTGCACCTG CCCCCAGGCG TCTGTCCCTG AGCAGTTTGC CTCCGTGCCC 4260

TGGAACTCCT TCAACCGCGA GGTGCTCAAG GCCCTGTACG GCTTCGCACC CATCTCCATG 4320

CACTGCAACA GGTCCTCGGC CGTCCGCTTC CAGGGCGAGT GGAATCGGCA GCCCCTGCCT 4380

GAGATCGTGT CCAGGTTCGA AGAGCCCACC CCTCACTGCC CAGCCAGCCA GGCTCAGAGC 4440

CCCGCCGGCC CCACCGTGCT GAACATCAGT GGGGGCAAAG GCTGAACGTG GGCGCCGC 4499

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIRES-074

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AAAGCGGCCG CCCCTCTCCC TCCCCCCCCC 30

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oZeocin-077

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAACTCGAGT CAGTCCTGCT CCTCGGCCAC 30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: 0IRES-Zeocin-075

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGTCAACTTG GCCATGGTTG TGGCAAGCTT 30

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: oIRES-Zeocin-076

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTGCCACAA CCATGGCCAA GTTGACCAGT 30

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5540 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: pOMCDACTH-IRES-THD-IRES-DBH-IRES-Zeocin

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..118

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 119..164

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 165..243

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 244..557

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 558..1155

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 1156..2166

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 2167..2766

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 2767..4560

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 4561..5159

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 5160..5534

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 5535..5540

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAGCTTGGTA CCGAGCTCGG ATCCACTAGT AACGGCCGCC AGTGTGCTGG AATTCTGCAG 60

ATATCCATCA CACTGGCGGC CGCGTCAGCC CTAGAGTCGA GCTGTGACGG TCCTTACAAT 120

GAAATGCAGC TGGGTTATCT TCTTCCTGAT GGCAGTGGTT ACAGGTAAGG GGCTCCCAAG 180

TCCCAAACTT GAGGGTCCAT AAACTCTGTG ACAGTGGCAA TCACTTTGCC TTTCTTTCTA 240

CAGGGGTGAA TTCGGCTTTC CCGGCCTTCC CCCTGGAGTT CAAGAGGGAG CTGACTGGCC 300

AGCGACTCCG GGAGGAGAT GGCCCCGACG GCCCTGCCGA TGACGGCGCA GGGGCCCAGG 360

CCGACCTGGA GCACAGCCTG CTGGTGGCGG CCGAGAAGAA GGACGAGGGC CCCTACAGGA 420

TGGAGCACTT CCGCTGGGGC AGCCCGCCCA AGGACAAGCG CTACGGCGGT TTCATGACCT 480

CCGAGAAGAG CCAGACGCCC CTGGTGACGC TGTTCAAAAA CGCCATCATC AAGAACGCCT 540

ACAAGAAGGG CGAGTGAGGG CACAGCGGGC CCCTCTCCCT CCCCCCCCCC TAACGTTACT 600

GGCCGAAGCC GCTTGGAATA AGGCCGGTGT GCGTTTGTCT ATATGTTATT TTCCACCATA 660

TTGCCGTCTT TTGGCAATGT GAGGGCCCGG AAACCTGGCC CTGTCTTCTT GACGAGCATT 720

CCTAGGGGTC TTTCCCCTCT CGCCAAAGGA ATGCAAGGTC TGTTGAATGT CGTGAAGGAA 780

GCAGTTCCTC TGGAAGCTTC TTGAAGACAA ACAACGTCTG TAGCGACCCT TTGCAGGCAG 840

CGGAACCCCC CACCTGGCGA CAGGTGCCTC TGCGGCCAAA AGCCACGTGT ATAAGATACA 900

CCTGCAAAGG CGGCACAACC CCAGTGCCAC GTTGTGAGTT GGATAGTTGT GGAAAGAGTC 960

AAATGGCTCT CCTCAAGCGT ATTCAACAAG GGGCTGAAGG ATGCCCAGAA GGTACCCCAT 1020

TGTATGGGAT CTGATCTGGG GCCTCGGTGC ACATGCTTTA CATGTGTTTA GTCGAGGTTA 1080

AAAAACGTCT AGGCCCCCCG AACCACGGGG ACGTGGTTTT CCTTTGAAAA ACACGATGAT 1140

AAGCTTGCCA CAACCATGGT CCCCTGGTTC CCAAGAAAAG TGTCGGAATT GGACAAGTGT 1200

CACCACCTGG TCACCAAGTT TGACCCTGAT CTGGACCTGG ACCACCCGGG CTTCTCTGAC 1260

CAGGTGTATC GCCAGCGTCG GAAGCTGATT GCAGAGATTG CCTTCCAGTA CAAGCACGGT 1320

GAACCAATTC CCCATGTGGA ATACACAGCG GAAGAGATTG CTACCTGGAA GGAGGTATAT 1380

GTCACGCTGA AGGCCTCTA TGCTACCCAT GCCTGCCGGG AGCACCTGGA GGGTTTCCAG 1440

CTTCTGGAAC GGTACTGTGG CTACCGAGAG GACAGCATCC CACAGCTGGA GGACGTGTCC 1500

CGCTTCTTGA AGGAGCGGAC TGGCTTCCAG CTGCGACCCG TGCCCGGTCT ACTGTCCGCC 1560

CGTGATTTTC TGGCCAGTCT GGCCTTCCGC GTGTTTCAAT GCACCCAGTA TATCCGCCAT 1620

GCCTCCTCAC CTATGCATTC ACCTGAGCCG GACTGCTGCC ATGAGCTGTT GGGACATGTA 1680

CCCATGTTGG CTGACCGCAC ATTTGCCCAG TTCTCCCAGG ACATTGGACT TGCATCTCTG 1740

GGGGCCTCAG ATGAAGAAAT TGAAAAACTC TCCACGGTGT ACTGGTTCAC TGTGGAATTC 1800

GGGCTATGTA AACAGAATGG GGAGCTGAAG GCTTATGGTG CAGGGCTGCT GTCTTCCTAC 1860

GGAGAGCTCC TGCACTCCCT GTCAGAGGAG CCTGAGGTCC GAGCCTTTGA CCCAGACACA 1920

GCAGCTGTGC AGCCCTACCA AGATCAAACC TACCAGCCTG TGTACTTTGT GTCCGAGAGC 1980

TTCAATGACG CCAAGGACAA GCTCAGGAAC TATGCCTCTC GTATCCAGCG CCCATTCTCT 2040

GTGAAGTTTG ACCCGTACAC ACTGGCCATT GACGTACTGG ACAGCCCTCA CACCATCCAG 2100

CGCTCCTTGG AGGGGGTCCA GGATGAGCTG CACACCCTGG CCCACGCACT GAGTGCCATT 2160

AGCTAAAATGC ATAGGATCCG CCCCTCTCCC TCCCCCCCCC CTAACGTTAC TGGCCGAAGC 2220

CGCTTGGAAT AAGCCCGGTG TGCGTTTGTC TATATGTTAT TTTCCACCAT ATTGCCGTCT 2280

TTTGGCAATG TGAGGGCCCG GAAACCTGGC CCTGTCTTCT TGACGAGCAT TCCTAGGGGT 2340

CTTTCCCCTC TCGCCAAAGG AATGCAAGGT CTGTTGAATG TCGTGAAGGA AGCAGTTCCT 2400

CTGGAAGCTT CTTGAAGACA AACAACGTCT GTAGCGACCC TTTGCAGGCA GCGGAACCCC 2460

CCACCTGGCG ACAGGTGCCT CTGCGGCCAA AAGCCACGTG TATAAGATAC ACCTGCAAAG 2520

GCGGCACAAC CCCAGTGCCA CGTTGTGAGT TGGATAGTTG TGGAAAGAGT CAAATGGCTC 2580

TCCTCAAGCG TATTCAACAA GGGGCTGAAG GATGCCCAGA AGGTACCCCA TTGTATGGGA 2640

TCTGATCTGG GGCCTCGGTG CACATGCTTT ACATGTGTTT AGTCGAGGTT AAAAAACGTC 2700

TAGGCCCCCC GAACCACGGG GACGTGGTTT TCCTTTGAAA AACACGATGA TAAGCTTGCC 2760

ACAACCATGT ACGGCACCGC GGTGGCGGTC TTCCTGGTCA TCCTCGTGGC TGCACTGCAG 2820

GGCTCGGCTC CCGCCGAGAG CCCCTTCCCC TTCCACATCC CCCTGGACCC CGAGGGGACC 2880

CTGGAGCTGT CCTGGAACAT CAGCTATGCG CAGGAGACCA TCTACTTCCA GCTCCTGGTG 2940

CGGGAGCTCA AGGCTGGTGT CCTGTTTGGG ATGTCGGACC GAGGGGAGCT GGAGAATGCT 3000

GACTTGGTGG TGCTCTGGAC TGACAGGGAC GGCGCCTACT TTGGGGATGC CTGGAGTGAC 3060

CAGAAGGGGC AGGTCCACCT GGACTCCCAG CAGGATTACC AGCTTCTGCG GGCACAGAGG 3120

ACTCCAGAAG GCCTGTACCT GCTCTTCAAG AGGCCTTTTG GCACCTGTGA CCCCAACGAC 3180

TACCTCATCG AGGACGGCAC CGTCCACCTG GTGTATGGAT TCCTGGAGGA GCGGCTCCGG 3240

TGGCTGGAGT CCATCAACAC ATCGGGCTTG CACACGGGGC TGCAGAGGGT GCAGCTGCTG 3300

AAGCCCAGCA TCCCCAAGCC GGCCCTGCCC GCGGACACGC GCACCATGGA GATCCGCGCC 3360

CCCGACGTCC TCATCCCCGG CCAGCAGACC ACGTACTGGT GCTACGTGAC CGAGCTCCCG 3420

GACGGCTTCC CCGCGCACCA CATCGTCATG TACGAGCCCA TCGTCACCGA GGGCAACGAG 3480

GCCCTGGTGC ACCACATGGA GGTCTTCCAG TGCGCCGCCG AGTTCGAGAC CATCCCCCAC 3540

TTCAGCGGGC CCTGCGACTC CAAGATGAAG CCGCAGCCGC TCAACTTCTG CGGTCACGTG 3600

CTGGCCGCCT GGGCCCTGGG CGCCAAGCCC TTTTACTACC CAGAGGAAGC AGGCCTGGCC 3660

TTCGGGGGGC CCGGCTCCTC CAGATTTCTC CGCCTGGAAG TTCACTACCA CAACCCACTG 3720

GTGATAACAG GCCGCGCGGA CTCCTCGGGC ATCCGCCTGT ACTACACGGC TGCGCTGCGG 3780

CGCTTCGACG CGGGCATCAT GGAGCTGGGC CTGGCGTACA CGCCCGTGAT GGCCATCCCC 3840

CCGCAGGAGA CGGCCTTCGT CCTCACCGGC TACTGCACGG ACAAGTGCAC CCAGCTGGCC 3900

CTGCCCGCCT CAGGGATTCA CATCTTCGCC TCTCAGCTCC ACACGCACCT GACCGGCGGG 3960

AAGGTGGTCA CAGTGCTGGC CAGGGACGGC CGGGAGACAG AGATCGTGAA CAGGGACAAC 4020

CACTACAGCC CACACTTCCA GGAGATCCGC ATGTTGAAGA AGGTCGTGTC TGTCCAGCGG 4080

GGAGACGTGC TCATCACCTC TTGCACATAC AACACGGAAG ACAGGAGGCT GGCCACCGTG 4140

GGGGGCTTCG GGATCCTGGA GGAGATGTGC GTCAACTATG TGCACTACTA CCCCCAGACG 4200

CAGCTGGAGC TCTGCAAGAG CGCCGTGGAC CCTGGCTTCC TGCACAAGTA CTTCCGCCTC 4260

GTGAACAGGT TCAACAGCGA GGAAGTCTGC ACCTGCCCCC AGGCGTCTGT CCCTGAGCAG 4320

TTTGCCTCCG TGCCCTGGAA CTCCTTCAAC CGCGAGGTGC TCAAGGCCCT GTACGGCTTC 4380

GCACCCATCT CCATGCACTG CAACAGGTCC TCGGCGGTCC GCTTCCAGGG CGAGTGGAAT 4440

CGGCAGCCCC TGCCTGAGAT CGTGTCCAGG TTGGAAGAGC CCACCCCTCA CTGCCCAGCC 4500

AGCCAGGCTC AGAGCCCCGC CGGCCCCACC GTGCTGAACA TCAGTGGGGG CAAAGGCTGA 4560

ACGTGGGCGG CCGCCCCTCT CCCTCCCCCC CCCCTAACGT TACTGGCCGA AGCCGCTTGG 4620

AATAAGGCCG GTGTGCGTTT GTCTATATGT TATTTTCCAC CATATTGCCG TCTTTTGGCA 4680

ATGTGAGGGC CCGGAAACCT GGCCCTGTCT TCTTGACGAG CATTCCTAGG GGTCTTTCCC 4740

CTCTCGCCAA AGGAATGCAA GGTCTGTTGA ATGTCGTGAA GGAAGCAGTT CCTCTGGAAG 4800

CTTCTTGAAG ACAAACAACG TCTGTAGCGA CCCTTTGCAG GCAGCGGAAC CCCCCACCTG 4860

GGGACAGGTG CCTCTGCGGC CAAAAGCCAC GTGTATAAGA TACACCTGCA AAGGCGGCAC 4920

AACCCCAGTG CCACGTTGTG AGTTGGATAG TTGTGGAAAG AGTCAAATGG CTCTCCTCAA 4980

GGGTATTCAA CAAGGGGCTG AAGGATGCCC AGAAGGTACC CCATTGTATG GGATCTGATC 5040

TGGGGCCTCG GTGCACATGC TTTACATGTG TTTAGTCGAG GTTAAAAAAC GTCTAGGCCC 5100

CCCGAACCAC GGGGACGTGG TTTTCCTTTG AAAAACACGA TGATAAGCTT GCCACAACCA 5160

TGGCCAAGTT GACCAGTGCC GTTCCGGTGC TCACCGCGCG CGACGTCGCC GGAGCGGTCG 5220

AGTTCTGGAC CGACCGGCTC GGGTTCTCCC GGGACTTCGT GGAGGACGAC TTCGCCGGTG 5280

TGGTCCGGGA CGACGTGACC CTGTTCATCA GCGCGGTCCA GGACCAGGTG GTGCCGGACA 5340

ACACCCTGGC CTGGGTGTGG GTGCGCGGCC TGGACGAGCT GTACGCCGAG TGGTCGGAGG 5400

TCGTGTCCAC GAACTTCCGG GACGCCTCCG GGCCGGCCAT GACCGAGATC GCGAGCAGC 5460

CGTGGGGGCG GGAGTTCGCC CTGCGCGACC CGGCCGGCAA CTGCGTGCAC TTCGTGGCCG 5520

AGGAGCAGGA CTGACTCGAG 5540

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 829 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: ProAKS

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..16

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 17..820

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 821..829

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCAAGCTTC GCCACCATGG CGCGGTTCCT GACACTTTGC ACTTGGCTGC TGTTGCTCGG 60

CCCCGGGCTC CTGCCGACCG TGCCGGCCGA ATGCAGCCAG GATTGCCGCA CGTGCAGCTA 120

CCGCCTAGTG CGCCCCGCCG ACATCAACTT CCTGGCTTGC GTAATGGAAT GTGAAGGTAA 180

ACTGCCTTCT CTGAAAATTT GGGAAACCTG CAAGGAGCTC CTGCAGCTGT CCAAACCAGA 240

GCTTCCTCAA GATGGCACCA GCACCCTCAG AGAAAATAGC AAACCGGAAG AAAGCCATTT 300

GCTAGCCAAA ACGTATGGGG GCTTCATGAA AAGGTATGGA GGCTTCATGA AGAAAATGGA 360

TGAGCTTTAT CCCATGGAGC CAGAAGAAGA GGCCAATGGA AGTGAGATCC TCGCCAAGCG 420

GTATGGGGGC TTCATGAAGA AGGATGCAGA GGAGGACGAC TCGCTGGCCA ATTCCTCAGA 480

CCTGCTAAAA GAGCTTCTGG AAACAGGGGA CAACCGAGAG CGTAGCCACC ACCAGGATGG 540

CAGTGATAAT GAGGAAGAAG TGAGCAAGAG ATATGGGGGC TTCATGAGAG GCTTAAAGAG 600

AAGCCCCCAA CTGGAAGATG AAGCCAAAGA GCTGCAGAAG CGATATGGGG GCTTCATGAG 660

AAGAGTAGGT CGCCCAGAGT GGTGGATGGA CTACCAGAAA CGGTATGGAG GTTTCCTGAA 720

GGGCTTTGCC GAGGCTCTGC CCTCCGACGA AGAAGGCGAA AGTTACTCCA AAGAAGTTCC 780

TGAAATGGAA AAAAGATACG GAGGATTTAT GAGATTTTAA GGATCCGGG 829

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 598 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
(B) CLONE: IRES sequence

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1..598

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAATTCCGCC CCTCTCCCTC CCCCCCCCCT AACGTTACTG GCCGAAGCCG CTTGGAATAA 60

GGCCGGTGTG CGTTTGTCTA TATGTTATTT TCCACCATAT TGCCGTCTTT TGGCAATGTG 120

AGGGCCCCGA AACCTGGCCC TGTCTTCTTG ACGAGCATTC CTAGGGGTCT TTCCCCTCTC 180

GCCAAAGGAA TGCAAGGTCT GTTGAATGTC GTGAAGGAAG CAGTTCCTCT GGAAGCTTCT 240

TGAAGACAAA CAACGTCTGT AGCGACCCTT TGCAGGCAGC GGAACCCCCC ACCTGGCGAC 300

AGGTGCCTCT GCGGCCAAAA GCCACGTGTA TAAGATACAC CTGCAAAGGC GGCACAACCC 360

CAGTGCCACG TTGTGAGTTG GATAGTTGTG GAAAGAGTCA AATGGCTCTC CTCAAGCGTA 420

TTCAACAAGG GGCTGAAGGA TGCCCAGAAG GTACCCCATT GTATGGGATC TGATCTGGGG 480

CCTCGGTGCA CATGCTTTAC ATGTGTTTAG TCGAGGTTAA AAAACGTCTA GGCCCCCCGA 540

ACCACGGGGA CGTGGTTTTC CTTTGAAAAA CACGATGATA AGCTTGCCAC AACCATGG 598

| Applicant's or agent's file reference number CTI/29 CIP PCT | International application No. |
|---|---|

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 54, line 5 14-23

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection

Address of depositary institution (including postal code and country)

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Identification Reference by Depositor: Cell Line, RINa/ProA/P030/P088

| Date of deposit | Accession Number |
|---|---|
| 07 June 1995 (07.06.95) | CRL 11921 |

C. ADDITIONAL INDICATIONS (leave blank if not applicable) — This information is continued on an additional sheet ☒

In respect of the designation of the EPO, samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application is refused or withdrawn or is deemed to be withdrawn, as provided in Rule 28(3) of the Implementing Regulations under the EPC only by the issue of a sample to an expert nominated by requester (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

EPO

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

| For receiving Office use only | For International Bureau use only |
|---|---|
| ☑ This sheet was received with the international application | ☐ This sheet was received by the International Bureau on: |
| Authorized officer: Yvette E. Gerstenzang | Authorized officer |

| Applicant's or agent's file reference number CTI/29 CIP PCT | International application No. |
|---|---|

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 54, line s 14-23

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution (including postal code and country)
12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Identification Reference by Depositor: Cell Line, RINa/ProA/P030/P088

| Date of deposit | Accession Number |
|---|---|
| 07 June 1995 (07.06.95) | CRL 11921 |

C. ADDITIONAL INDICATIONS (leave blank if not applicable) — This information is continued on an additional sheet ☒

In respect of the designation of Finland, until the application has been laid open to public inspection by the Finnish Patent Office, or has been finally decided upon by the Finnish Patent Office without having been laid open to public inspection, samples of the deposited microorganisms will be made available only to an expert in the art.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

Finland

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

| For receiving Office use only | For International Bureau use only |
|---|---|
| ☑ This sheet was received with the international application | ☐ This sheet was received by the International Bureau on: |
| Authorized officer | Authorized officer |

| Applicant's or agent's file reference number CTI/29 CIP PCT | International application No. |
|---|---|

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 54, line S 14-23

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
American Type Culture Collection

Address of depositary institution (including postal code and country)
12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Identification Reference by Depositor: Cell Line, RINa/ProA/P030/P088

| Date of deposit | Accession Number |
|---|---|
| 07 June 1995 (07.06.95) | CRL 11921 |

C. ADDITIONAL INDICATIONS (leave blank if not applicable) — This information is continued on an additional sheet ☐

Applicant(s) hereby give notice of my/our intention that samples of the above-identified culture shall be available only to experts in accordance with paragraph 3 of the Fourth Schedule to the Patents Rules 1995.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

Singapore

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

| For receiving Office use only | For International Bureau use only |
|---|---|
| ☑ This sheet was received with the international application | ☐ This sheet was received by the International Bureau on: |
| Authorized officer | Authorized officer |

WE CLAIM:

1. A cell stably transformed to produce at least one analgesic compound from each of the groups consisting of endorphins, enkephalins, and catecholamines.

2. The cell of claim 1, wherein the endorphin is ß-endorphin.

3. The cell of claim 1, wherein the enkephalin is met-enkephalin.

4. The cell of claim 1, wherein the catecholamine is norepinephrine or epinephrine.

5. The cell of any one of claims 1-4 wherein the cell is a RIN cell.

6. The cell of any one of claims 1-4 wherein the cell is an AtT-20 cell.

7. The cell of any one of claims 1-6 wherein the cell additionally produces a compound selected from the group consisting of galanin, somatostatin, neuropeptide Y, neurotensin, or cholecystokinin.

8. A cell transformed with a DNA encoding POMC, a DNA encoding TH, a DNA encoding DBH, and a DNA encoding ProA, each DNA molecule operably linked to an expression control sequence.

9. The cell of claim 8 wherein the cell is transformed with pCEP4-POMC-030, pcDNA3-hproA+KS-091, and pZeo-pCMV-rTHΔKS-IRES-bDBH-088.

10. The cell of claim 8 wherein the cell is transformed with pCEP4-h POMC-ΔACTH-032, pBS-CMV-proA, and pZeo-pCMV-rTHΔKS-IRES-bDBH-088.

11. The cell of claim 8 wherein the cell is transformed with pcDNA3-hPOMCDACTH-IRES-rTHD-IRES-bDBH-IRES-Zeocin-073 and pcDNA3-proA+KS-091.

12. A transformed cell producing at least one enkephalin, one endorphin and one catecholamine, wherein the cell is transformed with:

a first vector containing a DNA encoding POMC operably linked to an expression control sequence,

a second vector containing a DNA encoding pro-enkephalin A operably linked to an expression control sequence,

a third vector containing a DNA encoding TH operably linked to an expression control sequence and a DNA encoding dopamine beta hydroxylase operably linked to an expression control sequence.

13. A method for treating pain comprising implanting at an implantation site in a patient a therapeutically effective number of the cells of any of claims 1-12.

14. The method of claim 13 wherein the cells are encapsulated in a semi-permeable membrane to form a bioartificial organ.

15. The method of claim 14 wherein the bioartificial organ is immunoisolatory.

16. The method of any one of claims 13-15 wherein the implantation site is the CNS.

17. The method of any one of claims 13-15 wherein the implantation site is the sub-arachnoid space.

18. A method of producing a cell that secretes at least one enkephalin, one endorphin and one catecholamine, comprising transforming the cell with a DNA encoding POMC operably linked to a first expression control sequence, a DNA encoding pro-enkephalin A operably linked to a second expression control sequence, and a DNA encoding TH operably linked to a third expression control sequence and a DNA encoding dopamine beta hydroxylase operably linked to a fourth expression control sequence.

19. The method of claim 18 wherein said first, second, third and fourth expression control sequences are identical.

20. The use of the cells of any of claims 1-12 to manufacture a medicant for treatment of pain.

21. The cells of claim 20 wherein the cells are implanted.

22. The cells of any one of claims 21-22 wherein the cells are encapsulated in a semi-permeable membrane to form a bioartificial organ.

23. The cells of claim 22 wherein the bioartificial organ is immunoisolatory.

24. The cells of any one of claims 21-23 wherein the implantation site is the CNS.

25. The cells of any one of claims 21-23 wherein the implantation site is the sub-arachnoid space.

26. A bioartificial organ comprising:

(a) a biocompatible, permeable jacket surrounding a core; and

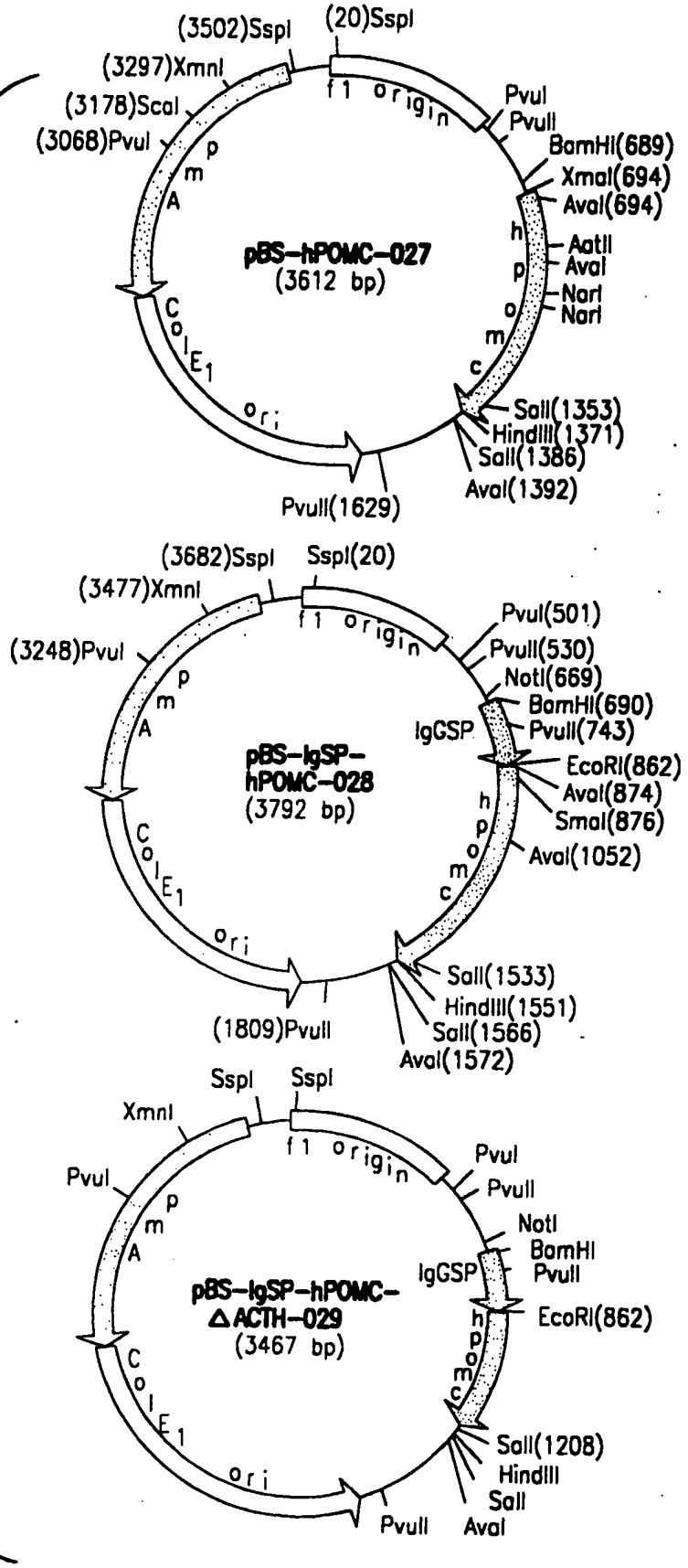
(b) said core comprising at least one living cell transformed to produce at least one analgesic compound from each of the groups consisting of endorphins, enkephalins, and catecholamines.

27. The bioartificial organ of claim 26 for use in treating pain.

28. A method of making a bioartificial organ comprising encapsulating a core comprising at least one living cell transformed to produce at least one analgesic compound from each of the groups consisting of endorphins, enkephalins, and catecholamines, with a biocompatible, permeable jacket.

29. The use of a bioartificial organ comprising the cells of claims 1-12 in manufacture of a medicament for treating of pain.

FIG. 1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(20)SspI
(3297)XmnI
(3178)ScaI
(3068)PvuI
f1 origin
PvuI
PvuII
BamHI(689)
XmaI(694)
AvaI(694)
AatII
AvaI
NarI
NarI
SalI(1353)
HindIII(1371)
SalI(1386)
AvaI(1392)
pBS-hPOMC-027
(3612 bp)
ColE1 ori
PvuII(1629)
(3682)SspI
SspI(20)
(3477)XmnI
(3248)PvuI
PvuI(501)
PvuII(530)
NotI(669)
BamHI(690)
PvuII(743)
IgGSP
EcoRI(862)
AvaI(874)
SmaI(876)
AvaI(1052)
pBS-IgSP-hPOMC-028
(3792 bp)
SalI(1533)
HindIII(1551)
SalI(1566)
AvaI(1572)
(1809)PvuII
SspI
SspI
XmnI
PvuI
PvuI
PvuII
NotI
BamHI
PvuII
IgGSP
EcoRI(862)
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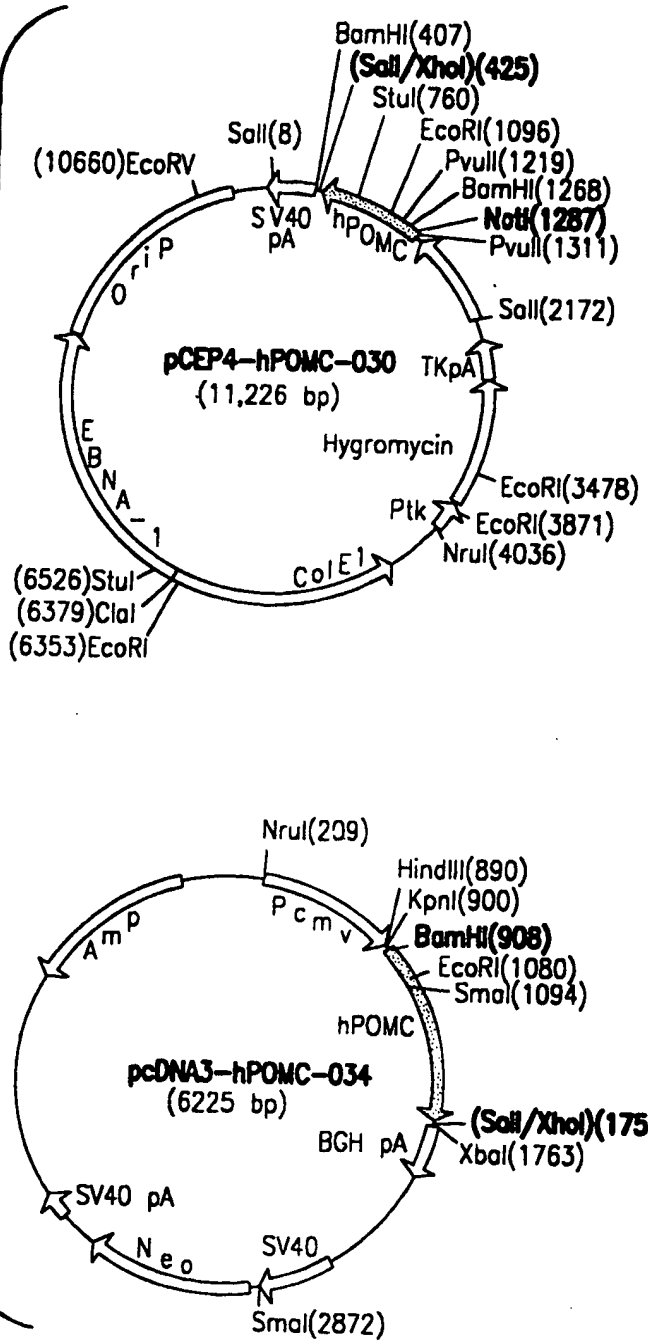
(3467 bp)
SalI(1208)
HindIII
SalI
AvaI
PvuII

FIG. 2a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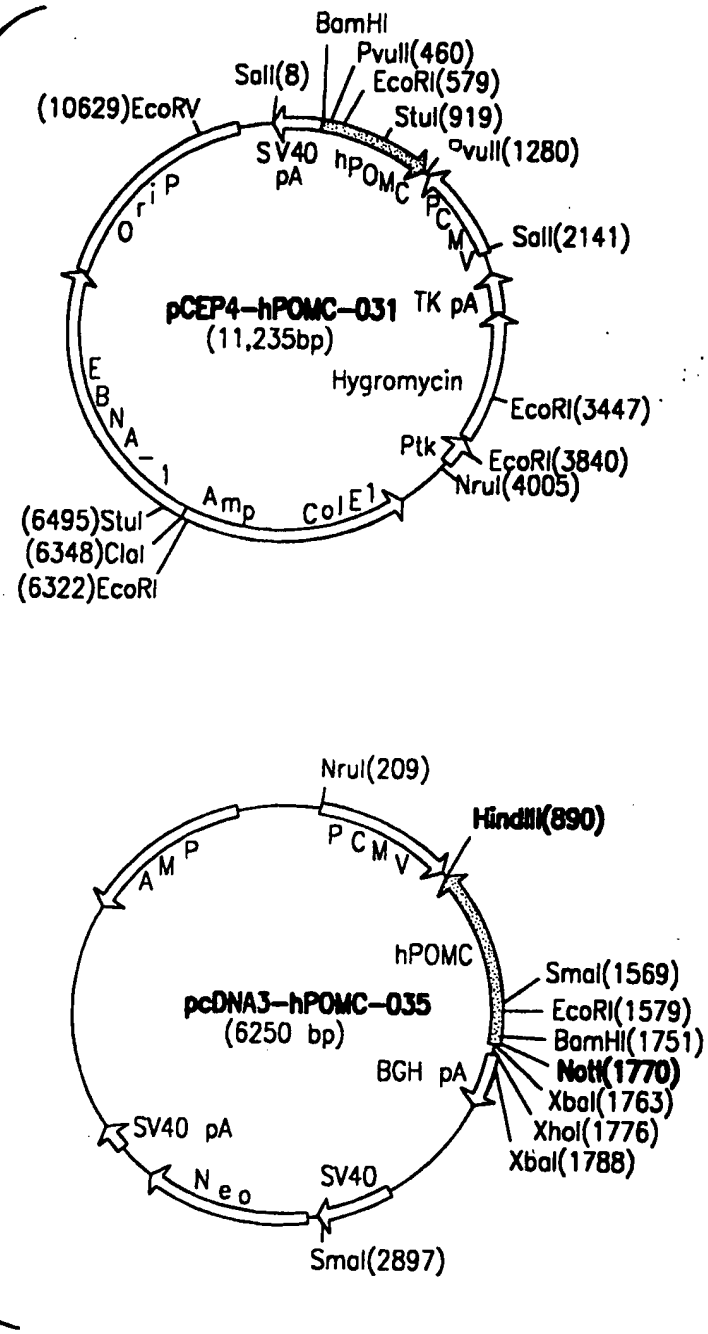
(SalI/XhoI)(425)
StuI(760)
SalI(8)
EcoRI(1096)
PvuII(1219)
(10660)EcoRV
BamHI(1268)
NotI(1287)
SV40 pA
hPOMC
PvuII(1311)
SalI(2172)
pCEP4-hPOMC-030
(11,226 bp)
TKpA
Hygromycin
EcoRI(3478)
Ptk
EcoRI(3871)
NruI(4036)
ColE1
(6526)StuI
(6379)ClaI
(6353)EcoRI
NruI(209)
HindIII(890)
KpnI(900)
BamHI(908)
EcoRI(1080)
SmaI(1094)
hPOMC
pcDNA3-hPOMC-034
(6225 bp)
(SalI/XhoI)(1756)
BGH pA
XbaI(1763)
SV40 pA
SV40
SmaI(2872)

FIG. 2b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EcoRI(579)
StuI(919)
PvuII(1280)
SalI(8)
(10629)EcoRV
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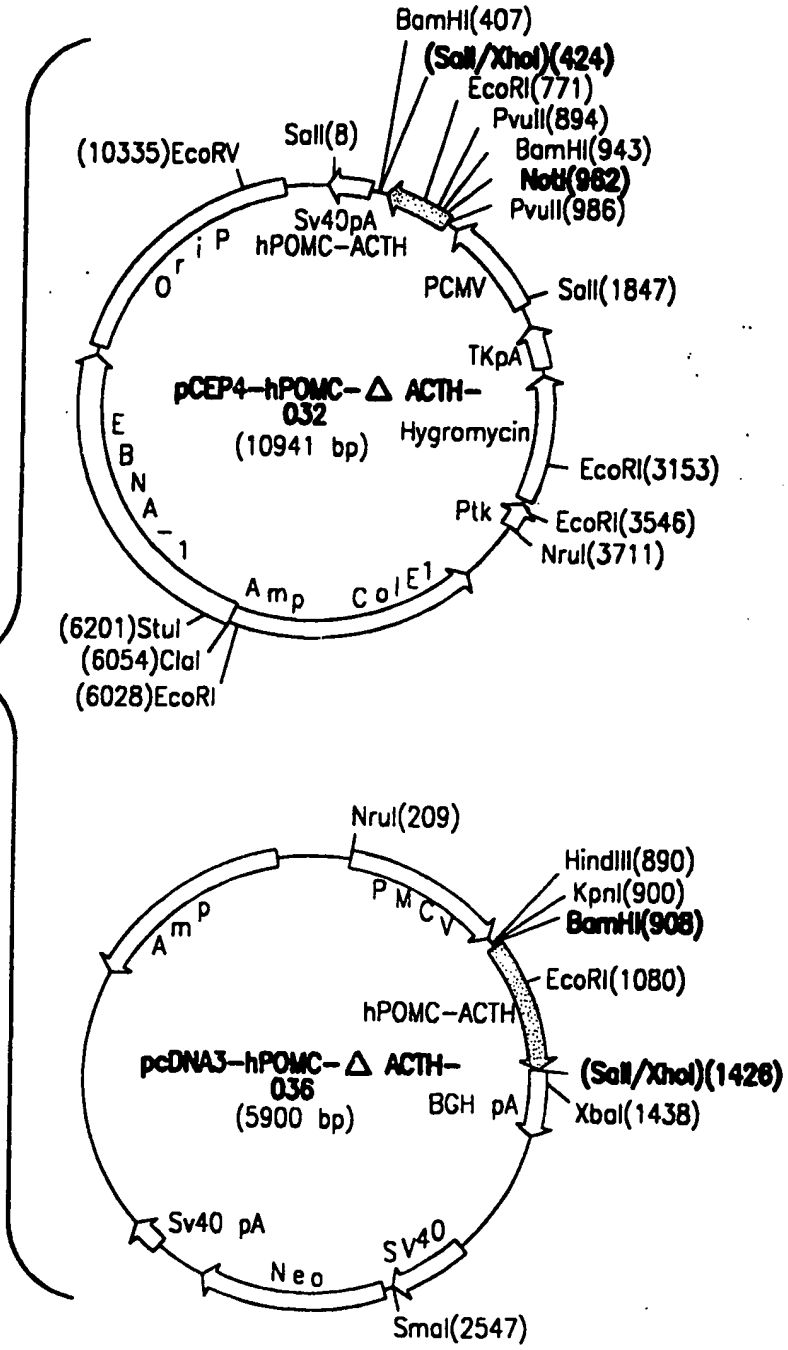
hPOMC
PCMV
SalI(2141)
Ori P
pCEP4-hPOMC-031
(11,235bp)
TK pA
Hygromycin
EcoRI(3447)
EBNA-1
Ptk
EcoRI(3840)
NruI(4005)
Amp
ColE1
(6495)StuI
(6348)ClaI
(6322)EcoRI
NruI(209)
HindIII(890)
PCMV
AMP
hPOMC
SmaI(1569)
EcoRI(1579)
BamHI(1751)
pcDNA3-hPOMC-035
(6250 bp)
BGH pA
NotI(1770)
XbaI(1763)
XhoI(1776)
XbaI(1788)
SV40 pA
Neo
SV40
SmaI(2897)

FIG. 3a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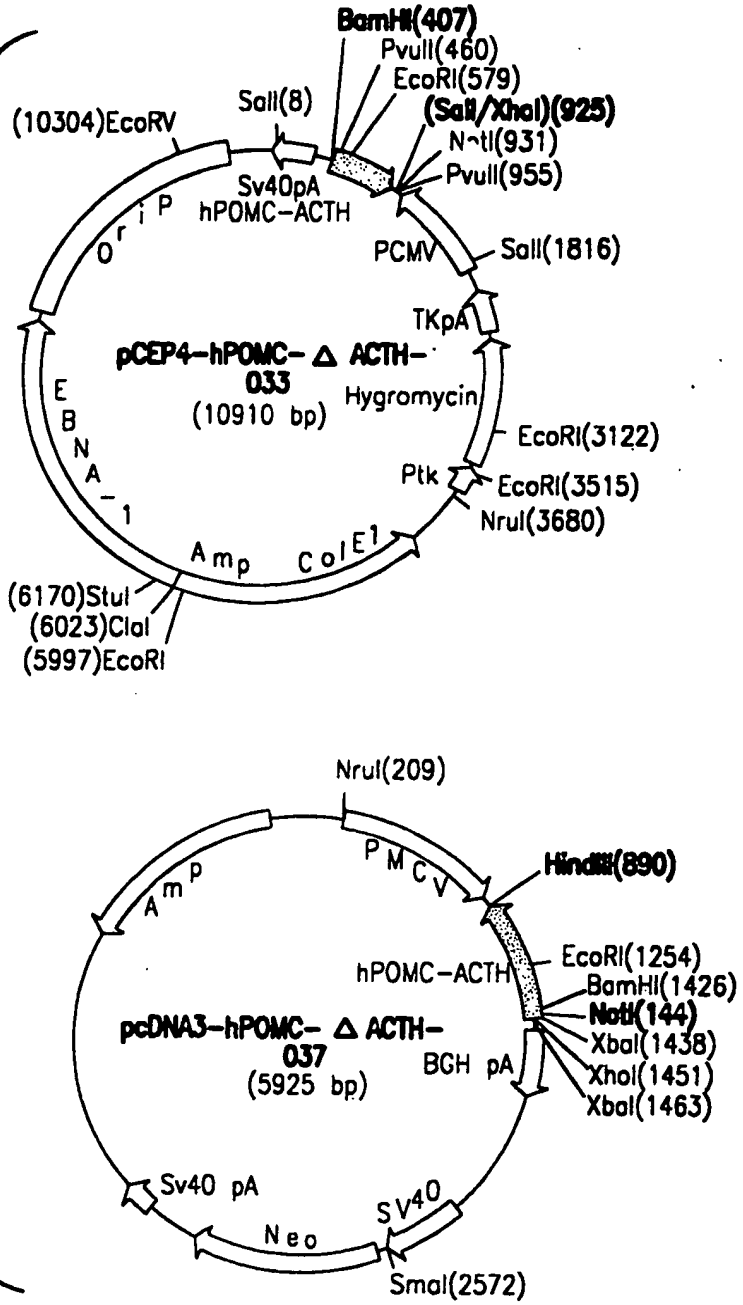
EcoRI(771)
PvuII(894)
BamHI(943)
NotI(982)
PvuII(986)
SalI(8)
(10335)EcoRV
Sv40pA
hPOMC-ACTH
Ori P
PCMV
SalI(1847)
TKpA
pCEP4-hPOMC-Δ ACTH-032
(10941 bp)
Hygromycin
EBNA-1
EcoRI(3153)
Ptk
EcoRI(3546)
NruI(3711)
Amp
ColE1
(6201)StuI
(6054)ClaI
(6028)EcoRI
NruI(209)
HindIII(890)
KpnI(900)
BamHI(908)
PMCV
Amp
EcoRI(1080)
hPOMC-ACTH
pcDNA3-hPOMC-Δ ACTH-036
(5900 bp)
(SalI/XhoI)(1426)
BGH pA
XbaI(1438)
Sv40 pA
SV40
Neo
SmaI(2547)

FIG. 3b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PvuII(460)
EcoRI(579)
(SalI/XhoI)(925)
NotI(931)
PvuII(955)
SalI(8)
(10304)EcoRV
Sv40pA
hPOMC-ACTH
Ori P
PCMV
SalI(1816)
TKpA
pCEP4-hPOMC-Δ ACTH-033
(10910 bp)
Hygromycin
EBNA-1
EcoRI(3122)
Ptk
EcoRI(3515)
NruI(3680)
Amp
ColE1
(6170)StuI
(6023)ClaI
(5997)EcoRI
NruI(209)
PMCV
HindIII(890)
Amp
hPOMC-ACTH
EcoRI(1254)
BamHI(1426)
NotI(144)
XbaI(1438)
XhoI(1451)
XbaI(1463)
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(5925 bp)
BGH pA
Sv40 pA
SV40
Neo
SmaI(2572)

FIG. 4

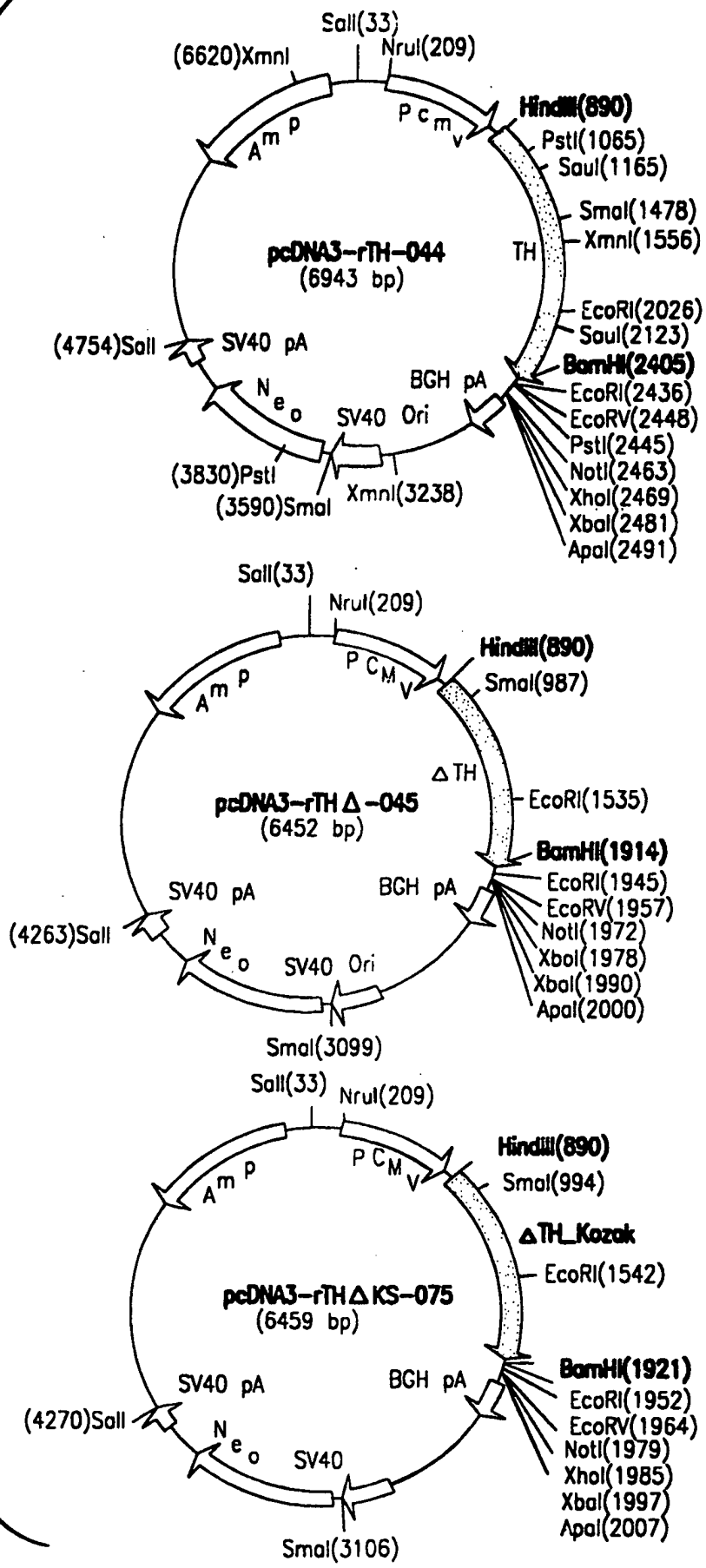

SalI(33)
NruI(209)
(6620)XmnI
Amp
PCMV
HindIII(890)
PstI(1065)
SalI(1165)
SmaI(1478)
XmnI(1556)
pcDNA3-rTH-044
(6943 bp)
TH
EcoRI(2026)
SalI(2123)
BamHI(2405)
(4754)SalI
SV40 pA
BGH pA
Neo
SV40 Ori
EcoRI(2436)
EcoRV(2448)
PstI(2445)
NotI(2463)
XhoI(2469)
XbaI(2481)
ApaI(2491)
(3830)PstI
(3590)SmaI
XmnI(3238)
SalI(33)
NruI(209)
HindIII(890)
SmaI(987)
Amp
PCMV
ΔTH
pcDNA3-rTHΔ-045
(6452 bp)
EcoRI(1535)
BamHI(1914)
SV40 pA
BGH pA
EcoRI(1945)
EcoRV(1957)
(4263)SalI
Neo
SV40 Ori
NotI(1972)
XbaI(1978)
XbaI(1990)
ApaI(2000)
SmaI(3099)
SalI(33)
NruI(209)
HindIII(890)
SmaI(994)
Amp
PCMV
ΔTH_Kozak
EcoRI(1542)
pcDNA3-rTHΔKS-075
(6459 bp)
BamHI(1921)
SV40 pA
BGH pA
EcoRI(1952)
(4270)SalI
Neo
SV40
EcoRV(1964)
NotI(1979)
XhoI(1985)
XbaI(1997)
ApaI(2007)
SmaI(3106)

FIG. 5

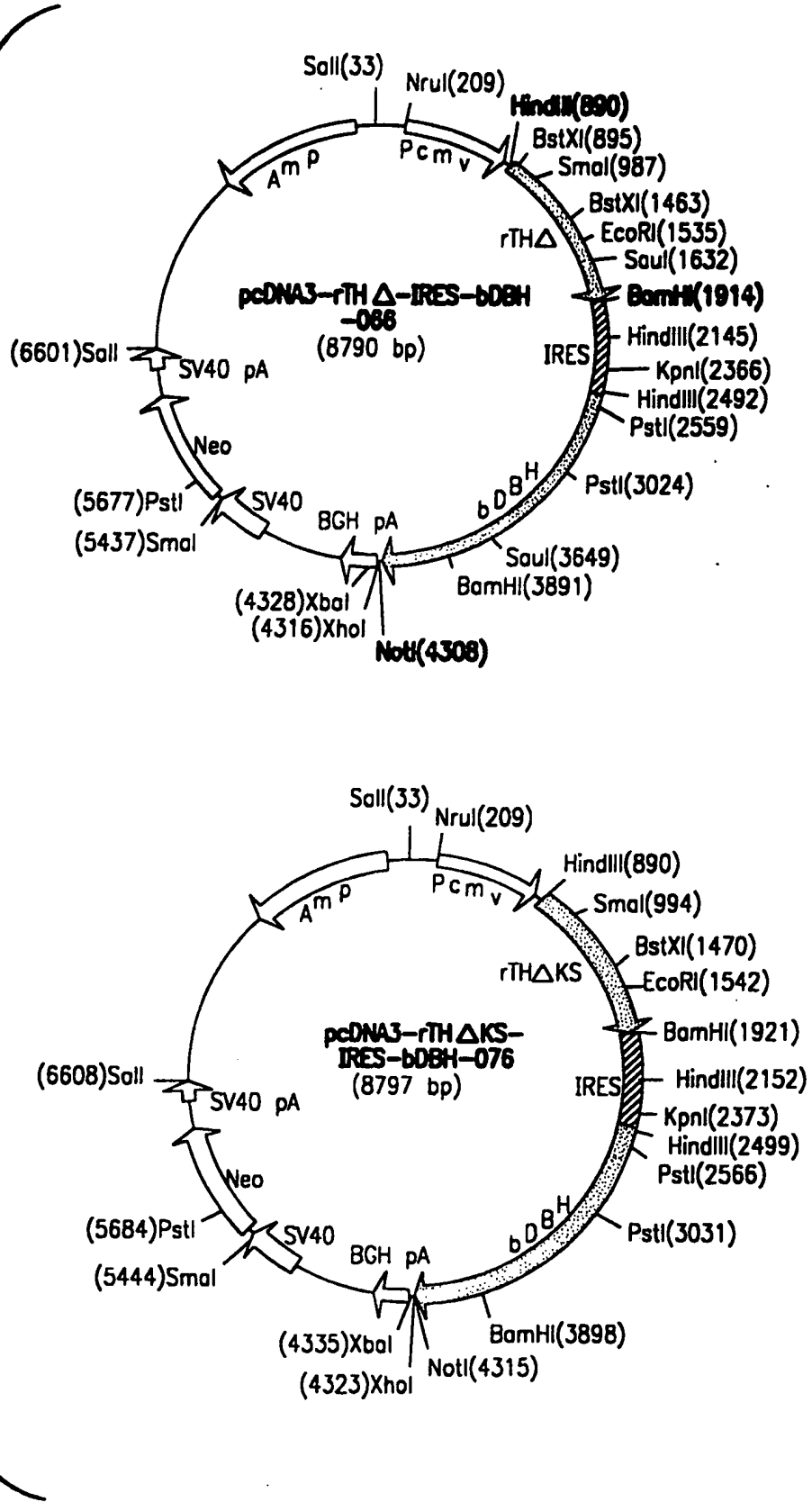

SalI(33)
NruI(209)
HindIII(890)
BstXI(895)
SmaI(987)
BstXI(1463)
EcoRI(1535)
SauI(1632)
BamHI(1914)
HindIII(2145)
KpnI(2366)
HindIII(2492)
PstI(2559)
PstI(3024)
SauI(3649)
BamHI(3891)
NotI(4308)
(4316)XhoI
(4328)XbaI
BGH pA
SV40
(5437)SmaI
(5677)PstI
Neo
SV40 pA
(6601)SalI
pcDNA3-rTHΔ-IRES-bDBH-066
(8790 bp)
rTHΔ
IRES
bDBH
Amp
Pcmv
SalI(33)
NruI(209)
HindIII(890)
SmaI(994)
BstXI(1470)
EcoRI(1542)
BamHI(1921)
HindIII(2152)
KpnI(2373)
HindIII(2499)
PstI(2566)
PstI(3031)
BamHI(3898)
NotI(4315)
(4323)XhoI
(4335)XbaI
BGH pA
SV40
(5444)SmaI
(5684)PstI
Neo
SV40 pA
(6608)SalI
pcDNA3-rTHΔKS-IRES-bDBH-076
(8797 bp)
rTHΔKS
IRES
bDBH
Amp
Pcmv

FIG. 6

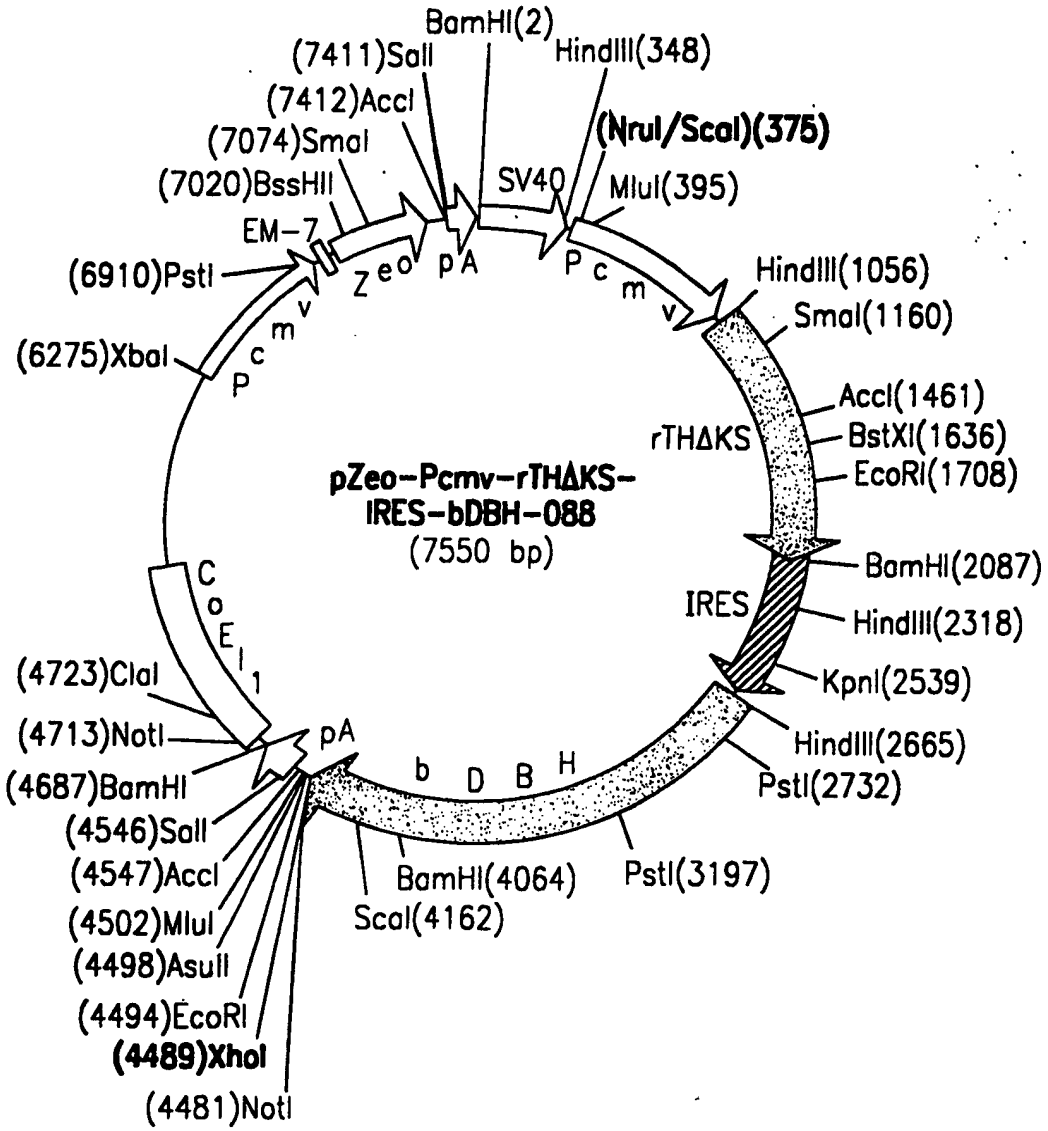

BamHI(2)
(7411)SalI
HindIII(348)
(7412)AccI
(NruI/ScaI)(375)
(7074)SmaI
(7020)BssHII
SV40
MluI(395)
EM-7
Zeo
pA
Pcmv
HindIII(1056)
(6910)PstI
SmaI(1160)
(6275)XbaI
AccI(1461)
rTHΔKS
BstXI(1636)
EcoRI(1708)
pZeo-Pcmv-rTHΔKS-
IRES-bDBH-088
(7550 bp)
BamHI(2087)
IRES
HindIII(2318)
KpnI(2539)
(4723)ClaI
(4713)NotI
pA
HindIII(2665)
(4687)BamHI
PstI(2732)
(4546)SalI
(4547)AccI
BamHI(4064)
PstI(3197)
(4502)MluI
ScaI(4162)
(4498)AsuII
(4494)EcoRI
(4489)XhoI
(4481)NotI

FIG. 7

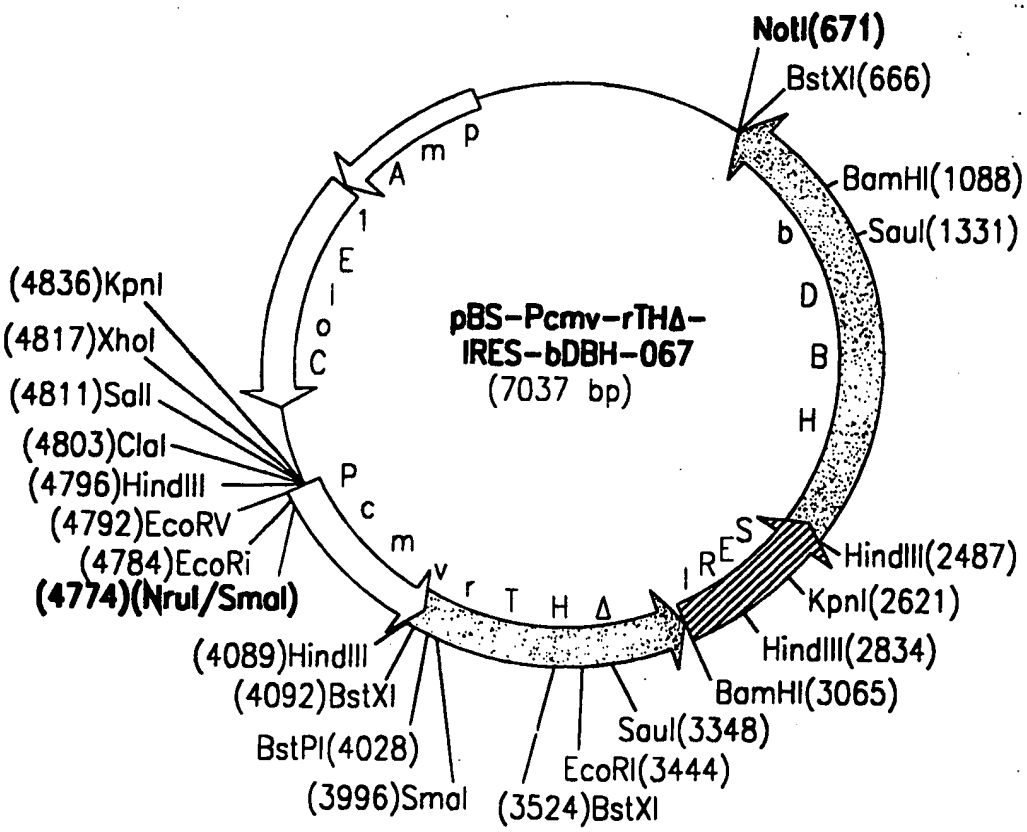
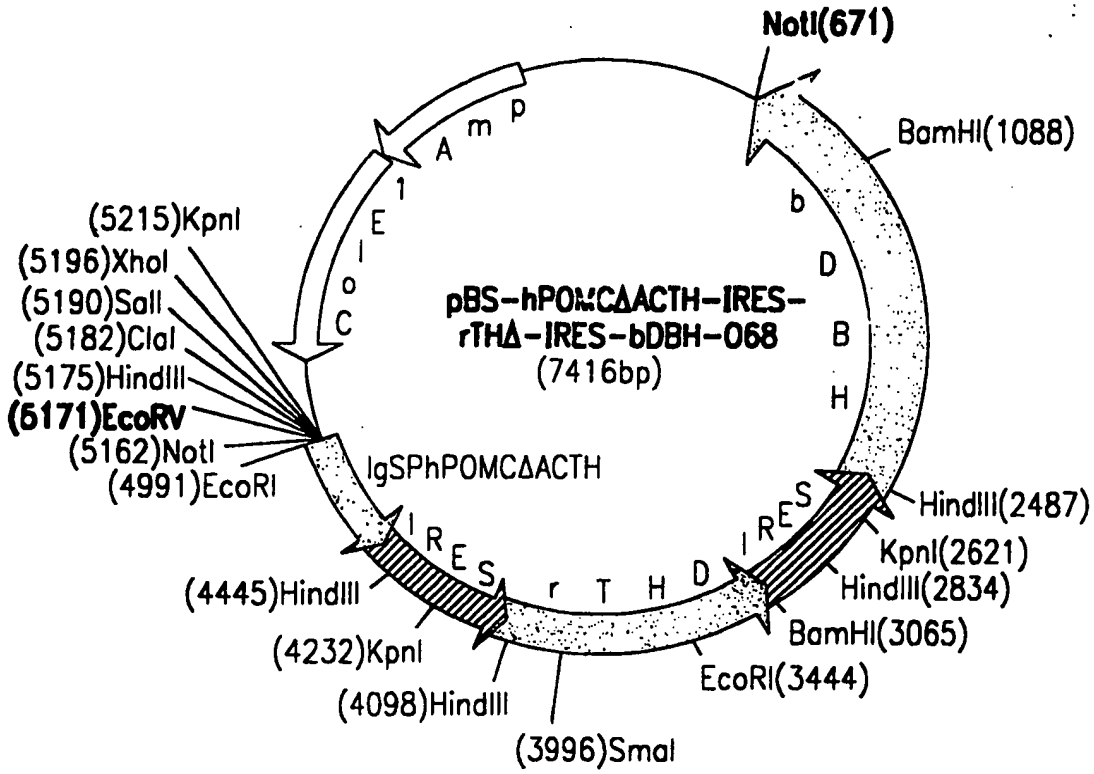

NotI(671)
BstXI(666)
BamHI(1088)
SauI(1331)
HindIII(2487)
KpnI(2621)
HindIII(2834)
BamHI(3065)
SauI(3348)
EcoRI(3444)
(3524)BstXI
(3996)SmaI
BstPI(4028)
(4092)BstXI
(4089)HindIII
(4774)(NruI/SmaI)
(4784)EcoRI
(4792)EcoRV
(4796)HindIII
(4803)ClaI
(4811)SalI
(4817)XhoI
(4836)KpnI
pBS-Pcmv-rTHΔ-
IRES-bDBH-067
(7037 bp)

FIG. 8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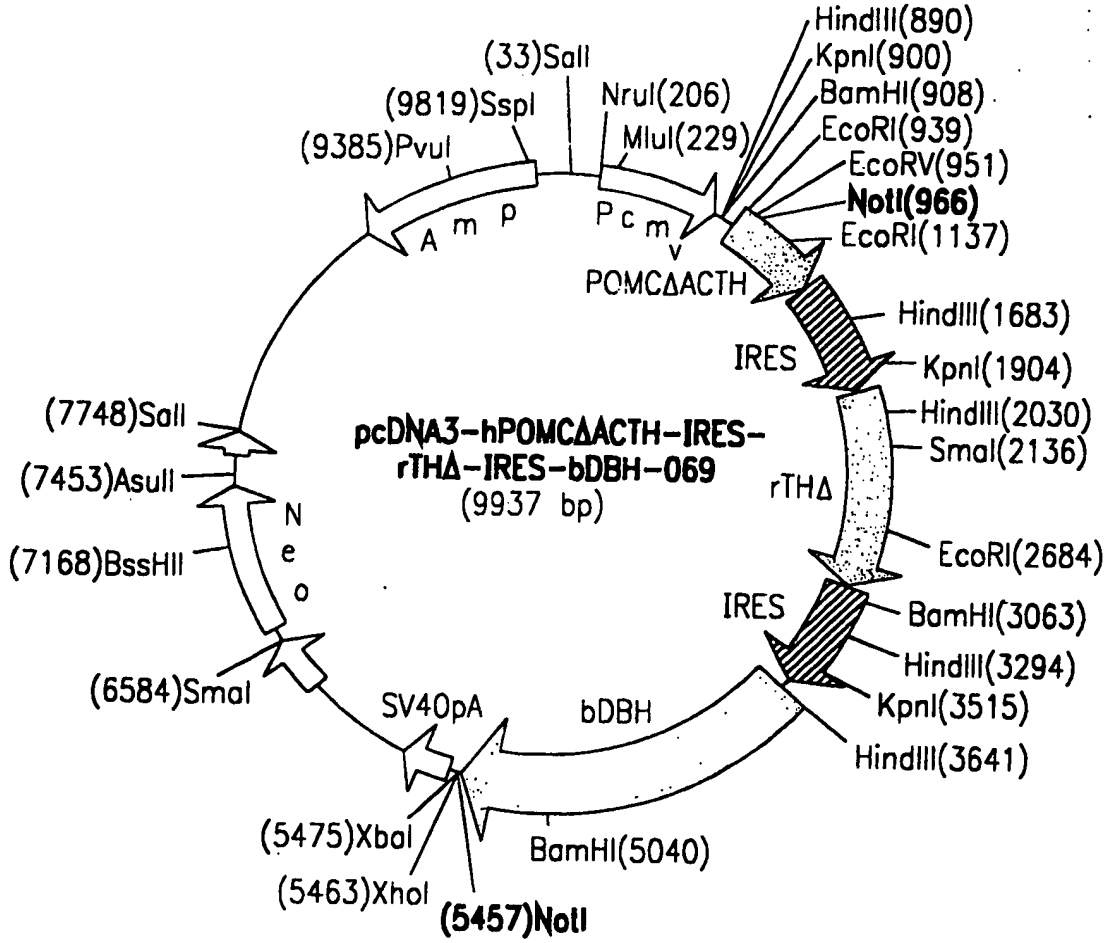
HindIII(2487)
KpnI(2621)
HindIII(2834)
BamHI(3065)
EcoRI(3444)
(3996)SmaI
(4098)HindIII
(4232)KpnI
(4445)HindIII
IgSPhPOMCΔACTH
(4991)EcoRI
(5162)NotI
(6171)EcoRV
(5175)HindIII
(5182)ClaI
(5190)SalI
(5196)XhoI
(5215)KpnI
pBS-hPOMCΔACTH-IRES-
rTHΔ-IRES-bDBH-068
(7416bp)

FIG. 9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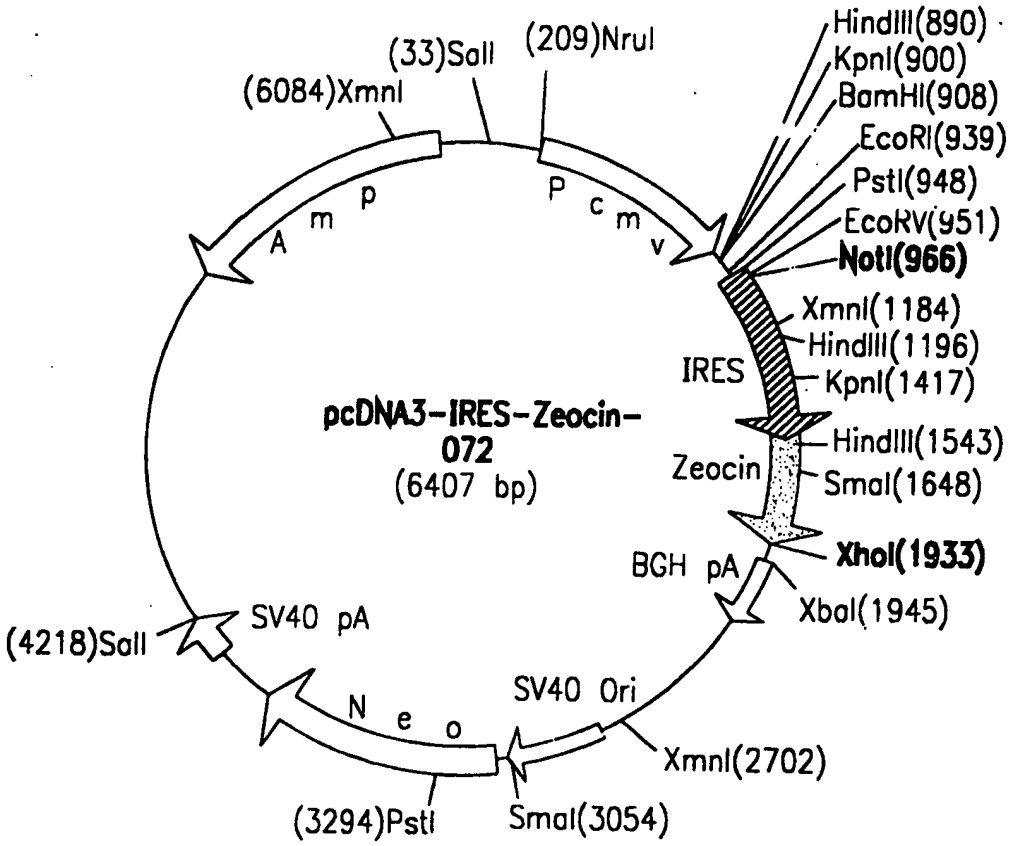
KpnI(900)
BamHI(908)
EcoRI(939)
EcoRV(951)
NotI(966)
EcoRI(1137)
(33)SalI
NruI(206)
MluI(229)
(9819)SspI
(9385)PvuI
Amp
Pcmv
POMCΔACTH
HindIII(1683)
IRES
KpnI(1904)
HindIII(2030)
SmaI(2136)
(7748)SalI
pcDNA3-hPOMCΔACTH-IRES-
rTHΔ-IRES-bDBH-069
(9937 bp)
rTHΔ
(7453)AsuII
(7168)BssHII
Neo
EcoRI(2684)
IRES
BamHI(3063)
HindIII(3294)
KpnI(3515)
HindIII(3641)
(6584)SmaI
SV40pA
bDBH
(5475)XbaI
BamHI(5040)
(5463)XhoI
(5457)NotI

FIG. 10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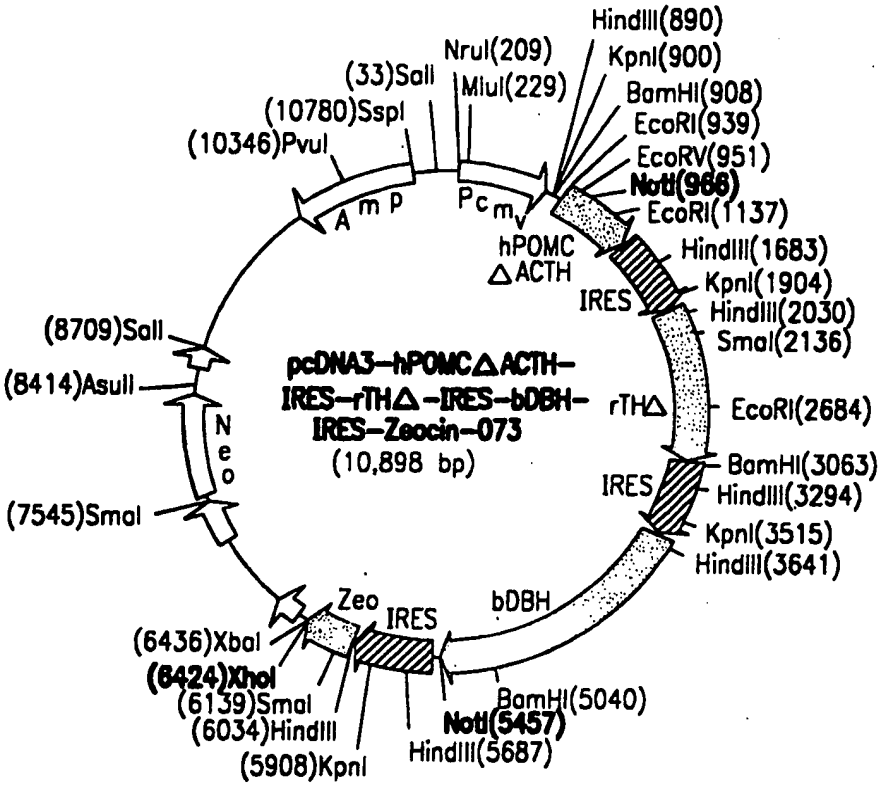
(209)NruI
(6084)XmnI
HindIII(890)
KpnI(900)
BamHI(908)
EcoRI(939)
PstI(948)
EcoRV(951)
NotI(966)
XmnI(1184)
HindIII(1196)
KpnI(1417)
IRES
HindIII(1543)
SmaI(1648)
Zeocin
XhoI(1933)
BGH pA
XbaI(1945)
pcDNA3-IRES-Zeocin-072
(6407 bp)
Amp
Pcmv
SV40 pA
(4218)SalI
Neo
SV40 Ori
XmnI(2702)
(3294)PstI
SmaI(3054)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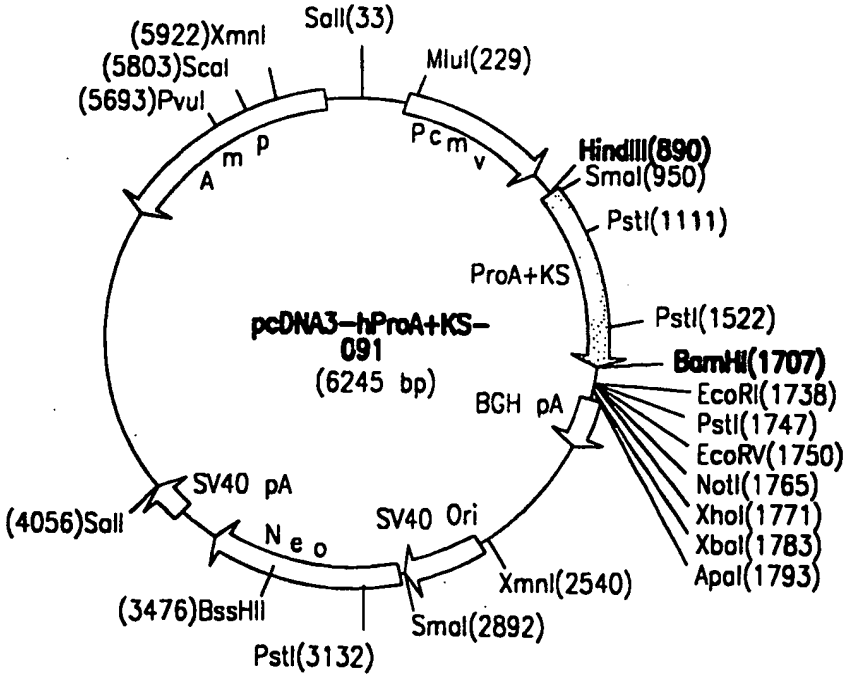
KpnI(900)
BamHI(908)
EcoRI(939)
EcoRV(951)
NotI(966)
EcoRI(1137)
HindIII(1683)
KpnI(1904)
HindIII(2030)
SmaI(2136)
EcoRI(2684)
BamHI(3063)
HindIII(3294)
KpnI(3515)
HindIII(3641)
BamHI(5040)
HindIII(5687)
NotI(5457)
(5908)KpnI
(6034)HindIII
(6139)SmaI
(6424)XhoI
(6436)XbaI
(7545)SmaI
(8414)AsuII
(8709)SalI
(10346)PvuI
(10780)SspI
(33)SalI
NruI(209)
MluI(229)
Amp
Pcmv
hPOMC
ΔACTH
IRES
rTHΔ
IRES
bDBH
Zeo
IRES
Neo
pcDNA3-hPOMCΔACTH-
IRES-rTHΔ-IRES-bDBH-
IRES-Zeocin-073
(10,898 bp)

FIG. 11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MluI(229)
HindIII(890)
SmaI(950)
PstI(1111)
PstI(1522)
BamHI(1707)
EcoRI(1738)
PstI(1747)
EcoRV(1750)
NotI(1765)
XhoI(1771)
XbaI(1783)
ApaI(1793)
XmnI(2540)
SmaI(2892)
PstI(3132)
(3476)BssHII
(4056)SalI
SV40 pA
SV40 Ori
BGH pA
ProA+KS
Amp
Pcmv
Neo
pcDNA3-hProA+KS-
091
(6245 bp)
(5693)PvuI
(5803)ScaI
(5922)XmnI

FIG. 12

# INTERNATIONAL SEARCH REPORT

| International Application No |
|---|
| PCT/US 96/09629 |

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/87 C12N5/10 A61K9/48 A61K38/16 A61K38/33

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO,A,95 05452 (CYTOTHERAPEUTICS, INC.) 23 February 1995 see the whole document, especially pages 12-31 and Example 6. --- | 1-4,8, 12-29 |
| A | J. NEUROSCI., vol. 14, 1994, pages 4806-4814, XP002018157 H.H. WU ET AL.: "Implantation of AtT-20 or genetically modified AtT-20/hENK cells in mouse spinal cord induced antinociception and opioid tolerance" cited in the application see the discussion. --- -/-- | 1 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 14 November 1996 | 28. 11. 96 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. ( + 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax ( + 31-70) 340-3016 | Yeats, S |

# INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 96/09629

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | PROC. NATL. ACAD. SCI. USA,<br>vol. 83, 1986,<br>pages 7522-7526, XP002018158<br>J. SAGEN ET AL.: "Analgesia induced by isolated bovine chromaffin cells implanted in rat spinal cord"<br>cited in the application<br>see the abstract and discussion.<br>--- | 1 |
| A | NATURE,<br>vol. 297, 1982,<br>pages 335-339, XP002018159<br>M. COCHET ET AL.: "Characterization of the structural gene and putative 5'-regulatory sequences for human proopiomelanocortin"<br>cited in the application<br>see the whole document.<br>----- | 1 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/09629

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13-17
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 13-17 are directed to a method for treatment of the human body by therapy (Rule 39 PCT), the search has been carried out based on the alleged effects of the composition mentioned in the claims.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

In .tional Application No
PCT/US 96/09629

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO-A-9505452 | 23-02-95 | AU-A- 7568094 | 14-03-95 |
| | | CA-A- 2169292 | 23-02-95 |
| | | FI-A- 960611 | 09-04-96 |
| | | NO-A- 960547 | 12-04-96 |